Carbon Fluxes from Decaying Beech Litter: Insights from a ^{13}C-Tracer Experiment and a New Method to Analyse the Stable Isotopes in Soil CO_2 Effluxes

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Summary

Forest soils contain large amounts of organic C, and thus are important potential sinks or sources of atmospheric CO_2. The C accumulation in forest soils is greatly driven by the rates at which C from aboveground plant litter is either mineralised to CO_2 or transported to the mineral soil via soil fauna and dissolved organic C (DOC). However, very few field studies, commonly using an isotopic tracer, have quantitatively assessed the different pathways of litter-derived C. Information is especially sparse for the decomposition of fine-woody litter, even though this litter type accounts for about 30% of the annual litter fall in temperate forests and, as it might mineralise more slowly than non-woody litter, could contribute substantially to the C pool in forest soils.

In my PhD research, I performed a tracer experiment in two forest soils (Rendzina and Cambisol) in the Swiss Jura mountains using ^{13}C-depleted beech litter (leaves and twigs). The main goal was to quantify the different pathways of litter-derived C for one year by tracking the labelled C into the CO_2 and DOC fluxes as well as into different fractions of the topsoil. In addition, I applied for the first time a new generation of spectrometer (QCLS: quantum cascade laser-based spectrometer) in a closed soil-chamber system to analyse the isotopic ratios of soil-respired CO_2 ($\delta^{13}C_{resp}$, $\delta^{18}O_{resp}$) at unique temporal resolutions.

The ^{13}C-tracer experiment revealed that the fate of the litter C depended on the 'quality' of the litter. While the woody twig litter decomposed largely *in situ* on the soil surface, large amounts of the leaf litter were transported to the mineral soil via soil fauna (~ 35% of the initial litter C). Moreover, the DOC leached from the twigs amounted only to half of that from the leaves throughout the year. However, the mineralisation rates differed little between the two litter types. After one year, the leaves had lost 29–34% of their initial C through CO_2 and the twigs 22–27%. This small difference between woody and non-woody litter is against the assumption of most soil C models. In combination, my findings suggest that twig litter is clearly less important for the C storage in these forest soils than leaf litter.

Although in deciduous forests leaf litter falls on the forest floor mostly at the beginning of the winter, we still know little about the decay and transport of this labile C pool during the cold season. My results show that the leaching of DOC from the ^{13}C-labelled litter occurred mainly in winter (~ 80%). By contrast, the C mineralisation during the five winter months contributed to 'only' 20–25% of the annual C losses from the litter through CO_2. This indicates that C mineralization and leaching of DOC from fresh litter are not basically linked. My tracer experiment also revealed that litter-derived CO_2 is a highly variable component of the winter soil respiration greatly driven by the air temperature. While on warm winter days

(T-air > 5°C) litter mineralisation accounted for up to 60% of the soil CO_2 effluxes, this CO_2 source was almost negligible on cold winter days (T-air < 1°C). On an annual scale, decaying leaf litter contributed to 10–12% of the soil CO_2 effluxes and twig litter 4–6%.

The DOC fluxes measured at three different depths (0, 5, 10 cm) were rather small as compared to other forest ecosystems. The leaching of DOC from the litter, for instance, contributed 6–10 times less to the total C loss from the litter than the C mineralisation. Nevertheless, in the long term, litter-derived DOC could be important for the storage of soil C in this forest ecosystem. I have several evidences that the litter DOC was strongly adsorbed on mineral surfaces, and thus was likely an important source of the 'new' C recovered in the heavy soil fraction (>1.6 g cm^{-3}) at 0–2 cm depth (2–4% of the initial litter C). This fraction is known to have much longer residence times in the soil than the light fraction. Moreover, I have evidence that a substantial part of the litter-derived DOC (20–40%) was rapidly mineralised by soil microbes. Together, physico-chemical interactions and biodegradation retained more than 90% of the DOC leached from the litter layer within the top centimetres of the mineral soil.

Laser spectroscopy is an emerging technique to analyse the isotopic composition of soil CO_2 effluxes *in situ* and at high temporal resolution. In collaboration with the EMPA, I employed the most recent spectrometer (QCLS) during a short field campaign. With a one-second interval, the QCLS measured the stable isotopes of CO_2 that accumulated in the headspace of a closed soil-chamber system. The unique resolution of the QCLS measurements allowed us to analyse non-linearities in the isotopic composition of CO_2 effluxes. This information was used to improve the estimation of $\delta^{13}C_{resp}$ and $\delta^{18}O_{resp}$.

To determine $\delta^{13}C_{resp}$, for instance, we used only the first 10 out of 20 minutes of CO_2 accumulation because there was a systematic shift in $\delta^{13}C_{resp}$ of 1.9‰ in the second part of the chamber measurements. This bias was probably the result of a ‘kinetic fractionation’ during CO_2 diffusion as it has recently been suggested by simulation studies, but so fare, has not been proven in field experiments. We estimated $\delta^{13}C_{resp}$ from both high resolution Keeling plots and directly from the ratio of the $^{12}CO_2$- and $^{13}CO_2$-flux rates. If the isotopic fluxes were derived from simple linear regression, the flux ratios were equal to the Keeling plot intercepts. The calculation of the fluxes with quadratic curve fits, however, resulted in, on average, 0.8‰ lower values for $\delta^{13}C_{resp}$. For the estimation of $\delta^{18}O_{resp}$, we used a new approach based on quadratic curve fits of the ^{18}O-Keeling plots. This was necessary as the $\delta^{18}O_{resp}$ increased immediately after the chamber system was closed probably due to the invasion of chamber

CO_2 into the first few centimetres of soil, where the ^{18}O of the CO_2 equilibrated partly with the soil water.

In summary, this thesis provides deep insights into the dynamic of both the decomposition of beech litter and the isotopic composition of soil CO_2 effluxes. It suggests rethinking the importance of fine-woody litter for the C storage in forest soils, and thus could contribute to improved soil C models. The evaluation of a QCLS in combination with a closed soil-chamber system clears the way for future studies that will use this new and promising method to determine the stable isotopes in soil-respired CO_2.

Kurzfassung

Waldböden enthalten grosse Mengen an organischem C und sind somit bedeutende potentielle Senken oder Quellen von atmosphärischem CO_2. Die Akkumulation von C in Waldböden wird wesentlich durch die Raten bestimmt, mit denen der C aus der oberirdischen Pflanzenstreu entweder durch mikrobielle Abbauprozesse als CO_2 veratmet wird oder mittels Bodenfauna und in gelöster Form (DOC) in den Mineralboden gelangt. Dennoch haben nur wenige Feldstudien, meist unter Verwendung einer isotopischen Markierung, die verschiedenen Flusswege von streubürtigem C quantitativ untersucht. Besonders wenig wissen wir über die Zersetzung von feiner Holzstreu, obwohl diese in temperierten Wäldern rund 30% des jährlichen Streufalls ausmacht und auf Grund ihrer langsamen Zersetzung erheblich zur C Speicherung in Waldböden beitragen könnte.

In meinem Forschungsprojekt führte ich in zwei Waldböden (Rendzina und saure Braunerde) des nördlichen Jura Gebirges ein Experiment mit ^{13}C-markierter Buchenstreu (Laub und Ästchen) durch. Während eines Jahres spürte ich den markierten C des sich zersetzenden Pflanzenmaterials in den CO_2 und DOC Flüssen sowie in verschiedenen Fraktionen des Oberbodens auf. Dies ermöglichte mir, die Flusswege des Streu C umfasssend zu quantifizieren. Zudem wendete ich erstmals einen neuartigen Spektrometer (QCLS: Quantum Cascade Laser-based Spectrometer) in Kombination mit einem geschlossenen Boden-Kammersystem an, um die Isotopensignatur der Bodenatmung ($\delta^{13}C_{resp}$, $\delta^{18}O_{resp}$) mit einer einmaligen zeitlichen Auflösung zu analysieren.

Das ^{13}C-Markierungsexperiment zeigte, dass der Verbleib des streubürtigen C von der Qualität der Streu abhing. Während die verholzten Ästchen weitgehend *in situ* an der Bodenoberfläche verrotteten, hatte die Bodenfauna nach einem Jahr grosse Mengen des Laubes (~ 35% des anfänglichen Streu C) in den Mineralboden transportiert. Des Weiteren wurde aus dem Laub rund doppelt so viel DOC ausgewaschen wie aus den Ästchen.

Hingegen unterschieden sich die Mineralisierungsraten der beiden Streuarten nur wenig. Nach einem Jahr hatte die Laubstreu 29–34% und die holzige Streu 22–27% ihrer anfänglichen C Menge über die Freisetzung von CO_2 verloren. Dieser Unterschied ist deutlich geringer als er für die Parametrisierung der meisten Boden C Modelle angenommen wird. Insgesamt legen meine Ergebnisse nahe, dass die feine Holzstreu für die C Speicherung in diesen Waldböden deutlich weniger wichtig ist als die Laubstreu.

Obwohl in Laubwäldern die Laubstreu hauptsächlich zu Beginn des Winters auf den Boden fällt, weiss man nur wenig über den Abbau und Transport dieses labilen C Pools während der kalten Jahreszeit. Meine Messungen und Modellierungen zeigen, dass die DOC Auswaschung aus der ^{13}C-markierten Streu vor allem im Winter erfolgte (~ 80%). Dagegen trug die C Mineralisierung während der fünf Wintermonate 'nur' 20–25% zum jährlichen C Verlust aus der Streu durch CO_2 bei. Mineralisierung und DOC Auswaschung aus frischer Streu scheinen also nicht grundsätzlich miteinander verknüpft zu sein. Mein Markierungsexperiment zeigte auch, dass das aus der Streuschicht stammende CO_2 ein hochvariabler Bestandteil der winterlichen Bodenatmung ist, wobei die Lufttemperatur eine entscheidende Rolle spielt. Während an warmen Wintertagen (T-Luft > 5°C) die Mineralisierung von frischer Streu bis zu 60% der Boden CO_2 Ausflüsse ausmachte, war diese CO_2 Quelle an kalten Wintertagen (T-Luft < 1°C) kaum nachweisbar. Über das ganze Jahr trug die verrottende Laubstreu 10–12% zur Bodenatmung bei und die holzige Streu 4–6%.

Die DOC Flüsse des untersuchten Buchenwaldes waren im Vergleich mit anderen Waldökosystemen eher gering. So trug die DOC Auswaschung 6–10 Mal weniger zum gesamten C Verlust aus der Streu bei als die C Mineralisierung. Für die langfristige Speicherung von Boden C in diesem Ökosystem könnte streubürtiges DOC gleichwohl von Bedeutung sein. Ich habe nämlich Indizien, dass das Streu DOC stark an Mineraloberflächen adsorbiert wurde und eine Hauptquelle des 'neuen' C war, der am Ende des Experimentes in der schweren Bodenfraktion (> 1.6 g cm^{-3}) in 0–2 cm Tiefe gespeichert war (2–4% des anfänglichen Streu C). Diese Fraktion hat bekanntlich deutlich längere Aufenthaltszeiten im Boden als die leichte Fraktion. Meine Resultate deuten auch darauf hin, dass ein bedeutender Teil des aus der Streu stammenden DOC (20–40%) rasch durch Bodenmikroorganismen mineralisiert wurde. Mit Hilfe der Isotopenmarkierung konnte ich zeigen, dass physikalisch-chemische Interaktionen und mikrobieller Abbau mehr als 90% des von der Streuschicht ausgewaschenen DOC innerhalb der ersten Zentimeter des Mineralbodens zurückhielten.

Laser Spektroskopie ist eine relativ neue und viel versprechende Technik, um die isotopische Zusammensetzung von Boden CO_2 Ausflüssen *in situ* und mit hoher zeitlicher

Auflösung zu analysieren. In Zusammenarbeit mit der EMPA setzte ich während einer kurzen Feldkampagne den neusten Spektrometer (QCLS) ein. Mit einem Intervall von einer Sekunde bestimmte der QCLS die Isotopensignatur des CO_2 ($\delta^{13}C$, $\delta^{18}O$), das sich im Gasraum eines geschlossenen Boden-Kammersystems akkumulierte. Die hohe Genauigkeit und Auflösung der QSLS Messungen ermöglichten uns, Nichtlinearitäten in der isotopischen Zusammensetzung der CO_2 Flüsse zu analysieren. Mit diesen Informationen konnten wir die Genauigkeit der $\delta^{13}C_{resp}$ und $\delta^{18}O_{resp}$ Schätzungen deutlich verbessern.

Um das $\delta^{13}C_{resp}$ zu bestimmen, verwendeten wir zum Beispiel nur die ersten 10 Minuten der 20-minütigen Kammermessungen, da wir im zweiten Teil der Messungen eine systematische Verschiebung des $\delta^{13}C_{resp}$ von 1.9‰ feststellten. Dieser Bias resultierte wahrscheinlich aus einer kinetischen Fraktionierung während der CO_2 Diffusion aus dem Boden, wie sie kürzlich in Simulationsstudien beschrieben, bis anhin aber noch nie in Feldexperimenten nachgewiesen werden konnte. Das $\delta^{13}C_{resp}$ schätzten wir sowohl aus hoch aufgelösten Keeling Plots als auch direkt aus dem Verhältnis der $^{12}CO2$ und $^{13}CO2$ Flussraten. Falls die isotopischen Flüsse durch lineare Regression ermittelt wurden, war das Flussratio identisch mit dem Achsenabschnitt des Keeling Plots. Die Berechnung der Flüsse mit einer quadratischen Kurvenanpassung führte dagegen zu durchschnittlich 0.8‰ tieferen Werten für das $\delta^{13}C_{resp}$. Für die Bestimmung des $\delta^{18}O_{resp}$ wendeten wir einen neuen Ansatz an, der auf einer quadratischen Kurvenanpassung der $\delta^{18}O$-Keeling plots beruht. Dies war notwendig, weil sich das $\delta^{18}O_{resp}$ unmittelbar nach der Schliessung der Bodenkammer stetig erhöhte. Wir vermuten, dass akkumuliertes CO_2 in die obersten Zentimeter des Bodens eindrang und dort teilweise die ^{18}O Signatur des Bodenwassers annahm, bevor es zurück in die Bodenkammer gelangte.

Die vorliegende Arbeit vermittelt tiefe Einblicke in die Dynamik sowohl der Zersetzung von Buchenstreu als auch der isotopischen Zusammensetzung von Boden CO_2 Ausflüssen. Sie zeigt auf, dass die Bedeutung von feiner Holzstreu für die C Speicherung in Waldböden überdenkt werden muss und könnte somit zu verbesserten Boden C Modellen beitragen. Die Evaluation eines QCLS in Kombination mit einem geschlossenen Kammersystem ebnet den Weg für zukünftige Studien, welche diese neue und viel versprechende Methode zur Bestimmung der stabilen Isotope in der Bodenatmung anwenden wollen.

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Abbreviations

COUP	Coupled Heat and Mass Transfer Model
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
EMPA	Swiss Federal Laboratories for Materials Science and Technology
HF	Heavy Fraction
IRGA	Infrared Gas Analyzer
IRMS	Isotope Ratio Mass Spectrometry
LF	Light Fraction
m a.s.l.	meters above sea level
PLFA	Phospholipids Fatty Acids
POM	Particulate Organic Matter
ppm	parts per million
QCLS	Quantum Cascade Laser-based Spectrometer
SOC	Soil Organic Carbon
SOM	Soil Organic Matter
TDLS	Tunable Diode Laser-based Spectrometer
WSL	Swiss Federal Institute of Forest, Snow and Landscape Research
$\delta^{13}C$, $\delta^{18}O$	Stable Isotope Ratio of C and O (delta notation)
$\delta^{13}C_{resp}$, $\delta^{18}O_{resp}$	Stable Isotope Ratio of C and O in soil-respired CO_2
f_{litter}	Fraction of labelled litter C in C fluxes and C pools

"A fact in itself is nothing.
It is valuable only for the idea attached to it,
or for the proof which it furnishes"

Claude Bernard

Part A Synopsis

In this part, I highlight the relevance of my PhD research and I present and combine the main findings of the three papers below, which are referred to in the text by their roman numerals:

I. Kammer A., Schmidt M. W. I., Hagedorn F. (2011) Decomposition pathways of ^{13}C-depleted leaf litter in forest soils of the Swiss Jura. Biogeochemistry, doi: 10.1007/s10533-011-9607-x.

II. Kammer A., Hagedorn F. (2011) Mineralisation, leaching and stabilisation of ^{13}C-labelled leaf and twig litter in a beech forest soil. Biogeosciences, 8, 2195–2208, doi:10.5194/bg-8-2195-2011.

III. Kammer A., Tuzson B., Emmenegger L., Knohl A., Mohn J., Hagedorn F. (2011) Application of a quantum cascade laser-based spectrometer in a closed chamber system for real-time $\delta^{13}C$ and $\delta^{18}O$ measurements of soil-respired CO_2. Agricultural and Forest Meteorology, 151, 39–48, doi:10.1016/j.agrformet.2010.09.001.

1. Introduction

1.1 Carbon fluxes from litter - why should we study them?

The relentless increase in atmospheric concentrations of CO_2 and other greenhouse gases is becoming one of the most important issues facing humanity in this century due to its impact on the global climate. Models predict global warming values by 2100 as large as 6°C compared to present for the highest emission scenarios (IPCC, 2007). One great uncertainty in these projections is how the global C cycle responds to altered climatic conditions (Washington et al., 2009). So far, both the oceans and terrestrial ecosystems might have considerably mitigated the CO_2 increase since only about half of the anthropogenic CO_2 emissions have accumulated in the atmosphere. However, it is very questionable whether this natural CO_2 sink will persist until the end of this century (Friedlingstein et al., 2006).

The forests in the northern hemisphere probably play a crucial role for the future concentration in CO_2 because they have been one of the largest terrestrial sinks of CO_2 over the last decades (Goodale et al., 2002). In particular, the C storage in forest soils has become a focus of research as soil organic matter (SOM) accounts for about 75% of the total stocks of organic C in temperate and boreal forests (Goodale et al., 2002; Pregitzer & Euskirchen, 2004). The dynamic of this large C pool is driven by the C inputs to soils originating mainly from above- (leaves, twigs etc.) and belowground (dead roots) plant litter and the release of CO_2 from decomposition of litter and SOM, the so-called heterotrophic soil respiration (Fig. 1). Only a slight imbalance between these fluxes, e.g. due to a different sensitivity to rising temperature, will significantly affect the atmospheric concentration of CO_2.

One of the problems research is facing is the pronounced heterogeneity of soil organic C (SOC) even at small scales (> 1 m), which complicates the detection of significant changes in this C pool (Heim et al., 2009). A profound understanding of the C cycling in forest soils is therefore indispensable for the modelling of current and future stocks of SOC over large areas (Paustian et al., 1997). However, today's soil C models probably only roughly represent the complexity of the C storage in soils primarily because the knowledge on the driving processes is still insufficient (Krull et al., 2003). In many forest ecosystems, for instance, it is still very uncertain how much of the incoming plant litter is either mineralised to CO_2, incorporated

into microbial biomass or transported to mineral soils through dissolved organic C (DOC) and via soil fauna (Fig. 1). Given this poor quantitative understanding of the pathways of decomposing litter, it is a great challenge to estimate the relative contribution of different tree species and plant parts (e.g. leaves, twigs, roots) to the organic C pool in forest soils (Crow et al., 2009). This limited information about the exact sources of SOC in turn makes it difficult, among other reasons, to reliably predict the C storage in soils, and thus to say whether this large C pool will be a sink or source of CO_2 in the near future.

In my thesis, I investigate the different pathways of decomposing litter in a beech forest in the Swiss Jura mountains and, based on this information, I also argue whether leaf or twig litter might be more important for the storage of organic C in these soils.

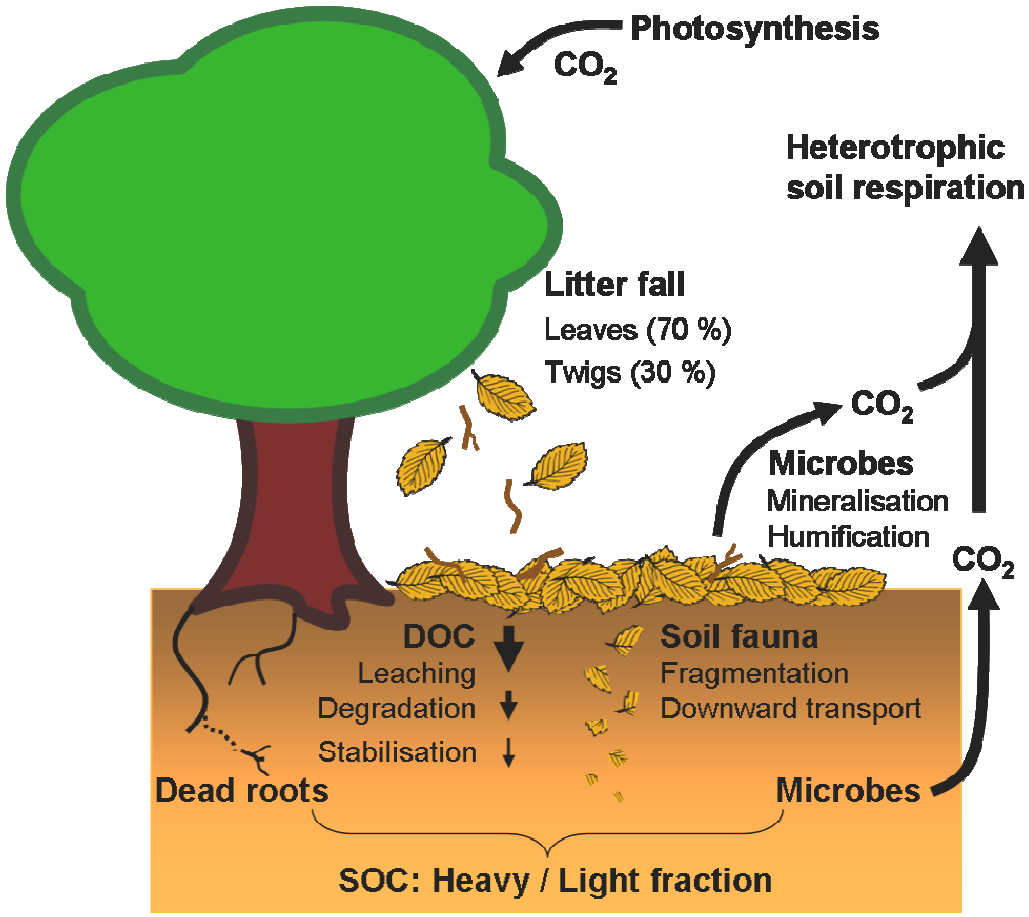

Figure 1. *Cycling of organic C in forest soils with focus on the pathways of decomposing litter and their underlying processes (own illustration). In the framework of this thesis, all the C fluxes and C pools illustrated have been investigated in a beech forest of the Swiss Jura apart from the root turnover and photosynthesis.*

1.2 The tracking of litter-derived C

The high demand for data on litter decomposition is reflected in the number of published articles on this topic, which has increased from 20 per year in the 80's to 2000 per year in our days (Prescott, 2010). The majority of these studies have analysed decomposition of litter either confined in mesh bags in the field or incubated in the lab (Cotrufo et al., 2010). Using these relatively simple and low-cost methods, the control of the mass loss from litter by litter quality and climatic conditions has been estimated for a large number of ecosystems (Moore et al., 1999; Liski et al., 2003; Hagedorn & Machwitz, 2007). Furthermore, the decay rates derived from such studies have provided the basis for the parameterisation of most soil C models (Liski et al., 2005; Larocque et al., 2006).

However, while litterbags and incubations are adequate methods to assess the decomposability of litter, they are inherently limited in analysing the different pathways of C loss from decomposing litter. They generally exclude a large part of the soil fauna from the decomposition process, and thus inhibit the fragmentation and downward transport of litter (Cotrufo et al., 2010). Moreover, the C loss from litter in mesh bags cannot entirely be attributed to microbial respiration since a substantial fraction (up to 30%) of the litter C is commonly leached as DOC (Magill & Aber, 2000; Hagedorn & Machwitz, 2007) and can remain in the soil for instance adsorbed on mineral surfaces (Kaiser & Guggenberger, 2000). This stabilised DOC might play a crucial role for the long-term storage of organic C in many forest soils (Kalbitz & Kaiser, 2008).

Deep insights into the fate of litter C, therefore, can probably be gained only in field studies using unconfined litter. Here, one approach is to compare total fluxes and pools of C in adjacent experimental plots with different amounts of litter input (Park et al., 2003; Rey et al., 2002; Sulzman et al., 2005). The difference between the plots is then attributed to the contribution of litter-derived C. However, these indirect estimates of C fluxes from recent litter are afflicted with uncertainty because: (1) the small-scale heterogeneity in CO_2 and DOC fluxes from 'old' SOC often masks the effect of added litter on the total fluxes of C; (2) the input or removal of litter has probably an effect on the microclimate at least in the top centimetres of the soil; (3) labile litter C may stimulate the mineralisation and leaching of ‘old’ SOC, often referred to as 'priming effect', which can lead to an overestimation of litter-derived C fluxes (Fontaine et al., 2007; Nottingham et al., 2008; Hagedorn et al., 2008).

An excellent way to overcome these uncertainties is the use of litter with an isotopic signature that differs distinctly from that of the native litter C for instance due to fumigation with isotopically enriched or depleted CO_2. In this approach, the isotope ratio ($\delta^{13}C$ or $\delta^{14}C$)

of the analysed C flux or C pool allows the fraction of litter-derived C to be determined reliably using simple mixing models. With help of this technique, several studies have estimated the contribution of recent litter to the soil respiration, to the DOC fluxes in organic layers and mineral soils, to the microbial community (PLFA) and to the SOC pool (see overview in Table 1).

Nevertheless, this powerful approach has been only applied in a small number of forest ecosystems and for a few litter types yet as it is relatively time-consuming and expensive. To my knowledge, for instance, no tracer experiment has investigated the C fluxes from decomposing fine-woody litter (twigs, branches, fruits). This litter type accounts for 30% of the annual litter fall in temperate forests (Thürig et al., 2005) and, given its low 'quality', it could contribute considerably to the C pool in forest soils (Vávřová et al., 2009). Moreover, most tracer studies have been performed in acidic forest soils (e.g. Subke et al., 2004; Fröberg et al., 2007), whereas soils with calcareous bedrock have largely been neglected. Litter decomposition in these soils, however, is of particular interest since large parts of the aboveground litter is incorporated into mineral soils within a few months due the high level of biological activity (Scheu, 1997). Finally, only a few field studies have taken into account several pathways of litter decomposition (see Fig. 1) to achieve a complete mass balance of the added litter (e.g. Rubino et al., 2010).

This thesis represents a comprehensive field study on the fate of litter-derived C (Table 1). Over one year, I tracked down the C of two different litter types (beech leaves and twigs) in the soil respiration, in the DOC and in different fractions of the mineral soil using ^{13}C as a tracer. In addition, the labelled litter experiment was performed in two soil types, in a slightly acidic soil and in a calcareous soil.

1.3 Challenges in the estimation of CO_2 effluxes from labelled litter

Beside the apparent strengths of the tracer approach, there are also some challenges associated with this method in particular regarding the estimation of the CO_2 release from isotopically labelled litter. In contrast to the DOC, the SOC and the microbial C, the isotopic signature of the soil-respired CO_2 ($\delta^{13}C$ of C3 plants = –35‰ to –20‰; Dawson et al., 2002) cannot directly be measured due to its dilution with atmospheric CO_2 ($\delta^{13}C$ = –8‰). Alternatively, it is often estimated from the change in the concentration and in the $\delta^{13}C$ of CO_2 during the

Table 1. Survey of studies that have tracked litter-derived C into different C fluxes and C pools of forest soils using ^{13}C or ^{14}C as a tracer. The completeness of this list is to the best of my knowledge. The isotopic label is expressed as either the isotope ratio (δ) of the litter or the difference (Δ) in the isotope ratio between the labelled litter and the native litter or SOM.

Site	Litter	Isotopic label	Measured C fluxes and pools	References
Wetzstein (D)	Norway spruce: needles	$\delta^{13}C = –36.2‰$	CO_2	Subke et al., 2004
Hesse (F)	Beech: leaves	$\delta^{13}C = –50.0‰$	CO_2	Ngao et al., 2005
Sakuragawa (J)	Aboveground litter: mainly oak	$\Delta^{13}C = 6.8‰$	CO_2	Sakata et al., 2007
Sierra Nevada (USA)	Ponderosa pine: needles, roots	$\Delta^{13}C = 2200‰$	CO_2, LF and HF of mineral soil	Bird & Torn, 2006; Bird et al., 2008
Asa (Sw)	Norway spruce: needles	$\delta^{13}C = –41.1‰$	DOC: litter layer, organic layer	Fröberg et al., 2007
Tuscania (I)	Poplar: leaves	$\Delta^{13}C = 160‰$	CO_2, mineral soil (0–20 cm), microbes (PLFA)	Rubino et al., 2010
Oak Ridge Reservation (USA)	Aboveground litter: mainly oak, maple and hickory	$\Delta^{14}C = 750‰$	CO_2, DOC: litter layer / organic layer / mineral soil, microbes (PLFA), LF and HF (0–30 cm)	Swanston et al., 2005; Cisneros-Dozal et al., 2006; Fröberg et al., 2009; Kramer et al., 2010
Lägeren (CH)	Beech: leaves, twigs	$\delta^{13}C_{leaf} = –40.8‰$ $\delta^{13}C_{twig} = –38.4‰$	CO_2, DOC: litter layer / mineral soil (5 and 10 cm), microbial biomass, LF and HF (0–2 cm)	This thesis

accumulation of soil-derived CO_2 in the headspace of a soil chamber. The $\delta^{13}C$ of the soil CO_2 efflux ($\delta^{13}C_{resp}$) is then given by the intercept of a simple linear regression through the plot of the ^{13}C ratios and the reciprocal values of the CO_2 concentrations, the so-called Keeling plot (Keeling, 1958).

The uncertainty of this extrapolation can greatly be reduced by allowing a CO_2 increase in the chamber of several hundred ppm, provided that the isotopic signature of the respired CO_2 is constant throughout the accumulation period (Ohlsson et al., 2005). The later, however, has recently been challenged by simulation studies, which showed that the non-steady-state conditions in closed soil chambers due to rising CO_2 concentrations may retard the diffusion of $^{12}CO_2$ more strongly than that of the heavier $^{13}CO_2$ (Risk & Kellman, 2008; Nickerson & Risk, 2009b). This mechanism called 'diffusive kinetic fractionation' would lead to an overestimation of the ^{13}C ratio of the soil CO_2 efflux. But to my knowledge, there is no study which has assessed this phenomenon and its implication for tracer studies thoroughly in the field.

Another critical point is the calculation of the cumulative CO_2 release from labelled litter. The temporal variability in litter-derived CO_2 effluxes is probably more pronounced than that of total CO_2 effluxes from soils due to the high lability of the litter C pool and the distinct temperature and moisture variability in soils at the surface (Borken et al., 2003; Cisneros-Dozal, 2006; Joos et al., 2010). However, this variability is often insufficiently covered since effort and costs for measuring $\delta^{13}C_{resp}$ allow only a limited number of sampling days. So far, most tracer studies have estimated the total C losses from litter through CO_2 by interpolating the flux rates between few measurements without taking temperature and moisture variations into consideration (e.g. Ngao et al., 2005; Bird & Torn, 2006). This can easily result in significant under- or overestimations of the cumulated CO_2 effluxes. Therefore, new approaches are required to improve gap filling between temporary CO_2 effluxes from labelled litter and, even more desirable, to increase the accuracy and frequency of $\delta^{13}C_{resp}$ analyses.

*In my PhD research, I developed a new model which uses temperature and declining decomposability of litter C to improve the estimates for the cumulative C loss from litter through CO_2 (**Paper I** and **II**). Furthermore, I employed a new instrument which can make high frequency measurements of the stable isotopes of CO_2 accumulating in soil chambers (**Paper III**, see also the next chapter). These measurements allowed the examination of non-linearity in the isotopic effluxes of soil CO_2.*

1.4 New methods to determine the stable isotopes in soil CO_2 effluxes

The most promising technique to analyse the stable isotopic composition of soil-respired CO_2 at high temporal resolution is laser spectroscopy. While the offline measurements with isotope ratio mass spectrometry (IRMS) greatly limit the frequency of the sampling, laser based spectrometers allow the isotopic composition of CO_2 to be analysed continuously in the field over several days or even weeks (Bowling et al., 2003). Recently, this relatively new technique has been employed in several field experiments to assess the daily and seasonal variation in $\delta^{13}C_{resp}$ (Bahn et al., 2009; Marron et al., 2009; Moyes et al., 2010). All of these studies coupled a mid-IR tunable diode laser spectrometer (TDLS) with an open chamber system. However, I am not aware of any published data on the application of laser spectroscopy in a closed soil-chamber system, even though this type of chamber has been the most frequently used method in combination with IRMS measurements (Ohlsson et al., 2005). Closed-chamber systems require a less complex chamber design and are, in particular, easier to handle in the field than open chambers. On the other hand, the open chamber technique ensures steady state conditions between chamber headspace and soil for several minutes. This may reduce chamber-to-soil feedbacks (Pumpanen et al., 2004; Midwood et al., 2008).

The newest instruments for isotopic measurements of CO_2 are quantum cascade laser-based spectrometers (QCLS) which have a higher performance than TDLS. The prototype of the QCLS, designed and developed at the Swiss federal laboratories for materials science and technology (EMPA), has recently been used to measure the isotopic composition of ambient CO_2 at a grass land site and at the Jungfraujoch (3580 m a.s.l.) (Tuzson et al., 2008 and 2010). However, the QCLS has not been employed and evaluated yet in any soil respiration study in the field. Given the high precision and temporal resolution of the QCLS measurements, they could provide insights into the dynamics of CO_2 and its stable isotopes during accumulation in soil chambers, and thus may help to reduce the uncertainty in estimates for $\delta^{13}C_{resp}$ and $\delta^{18}O_{resp}$.

*My thesis presents the first application of a QCL-based spectrometer in a closed-soil chamber system to measure the isotopic composition of soil-respired CO_2 (**Paper III**).*

2. Objectives

The superior aims of this thesis are to improve the understanding of beech litter decomposition in calcareous forest soils and to evaluate a new system based on laser spectroscopy to make high frequency measurements of the stable isotopic composition of soil-respired CO_2.

More precisely, the research objectives are to assess

- the fate of beech litter during one year of decomposition. How much of the litter C is released as CO_2, leached as DOC, incorporated into the microbial biomass and transported to the mineral soil?
- how the pathways of C loss differ between leaves and twigs and what this implies for the relative contribution of both litter types to the SOC pool
- short-term and annual contributions of decaying beech litter to C fluxes and C pools
- whether QCL-based spectrometers can be applied in closed soil-chamber systems to determine short-term variations in $\delta^{13}C_{resp}$ and $\delta^{18}O_{resp}$

3. Materials and Methods

3.1 Study site and labelled litter experiment

The litter manipulation experiment was conducted in a mixed beech forest on the south-facing slope of the Lägeren mountain (680 m a.s.l.). The Lägeren research site (CH-Lae) contributes to the CarboEurope IP, which is a network to monitor the CO_2 exchange in terrestrial ecosystems across Europe. The site was particularly convenient for the aims of this study since it is close to Zurich (~ 20 km), provides a well-developed infrastructure (meteorological station, air-conditioned cottage etc.) and, as it belongs to the most north-eastern part of the Swiss Jura, allows the investigation of soil C dynamics in base-rich soils. Moreover, it offered the opportunity to join other research projects (e.g. Heim et al., 2009; Ruehr et al., 2010).

The litter experiment was performed on two soil types 200 m apart. One of the soils was a Rendzic Leptosol (or Rendzina; pH = 7.5) and the other a Haplic Cambisol (pH = 5.9). At the beginning of the cold season 2007, ^{13}C-depleted leaves ($\delta^{13}C$ = –40.8‰) and twigs ($\delta^{13}C$ = –38.4‰) were added to the soils. The litter originated from four year old beech trees of a CO_2 fumigation experiment at the WSL (Hagedorn et al., 2005). To recognize the isotopic signal for both litter types equally well, I added larger amounts of twigs (2 kg m^{-2}) than of leaves (0.75 kg m^{-2}) because I expected that the woody litter would decompose much more slowly than the leaf litter. One third of the experimental plots were covered with polystyrene shreds instead of litter ('bare-soil treatment'). The plots were equipped to measure the soil CO_2 effluxes and the DOC leaching below the litter layer as well as at depths of 5 and 10 cm (Fig. 2). In addition, there was a free area for repeatedly taking soil and litter samples. To amplify the ^{13}C signal of litter-derived CO_2, root respiration was minimized by digging a 30 cm deep trench around each plot.

3.2 Sampling, analyses and calculations

For one year, the soil CO_2 effluxes were measured at bi-weekly intervals. The contribution of the litter to soil CO_2 effluxes was determined on ten sampling days. Soil water was permanently collected and then pooled on a monthly base. Soil samples were analysed for the

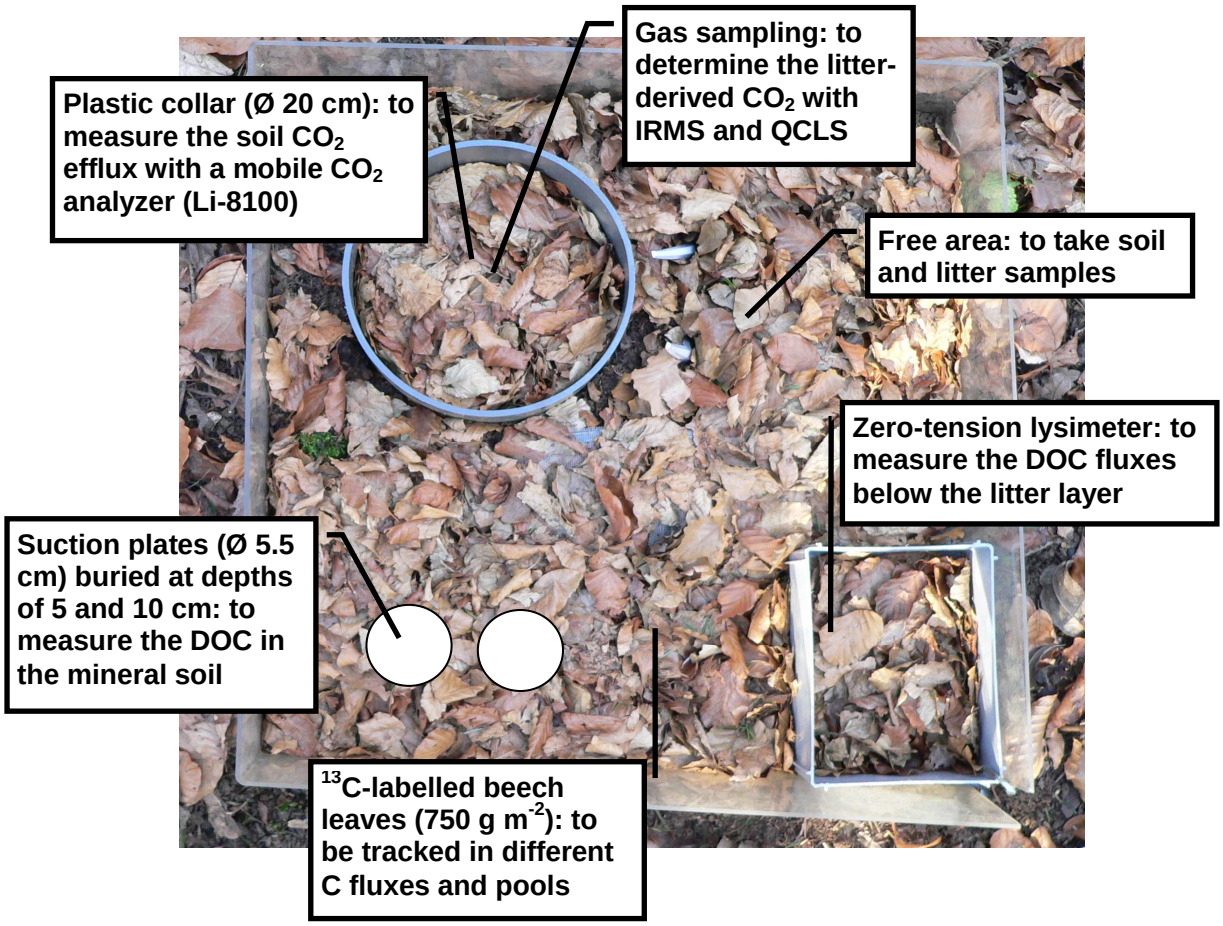

Figure 2. *Bird's-eye view on a plot (50 × 50 cm) of the litter experiment with its different installation to follow the fate of the litter-derived C.*

microbial biomass with the chloroform-fumigation extraction and physically fractionated into the light (< 1.6 g cm^{-3}) and heavy soil fraction. In freeze-dried water and milled soil samples, both the concentration and the ^{13}C ratio of the organic C were measured with an elemental analyzer coupled to an IRMS. The stable isotopes are expressed in the delta (δ) notation throughout the thesis which is defined as:

$$\delta_{sample} = [R_{sample} / R_{standard} - 1] \times 1000‰ \quad (1)$$

where R is the molar ratio of the heavy to the light isotope. The limestone Vienna PeeDee belemnite (V-PDB) is used as a standard for C and has a $^{13}C/^{12}C$ ratio of 0.011237. The contribution of the labelled-litter C (f_{litter}) to the fluxes and pools of soil C was calculated using the following mixing model:

$$f_{litter} = (\delta^{13}C_{soil+litter} - \delta^{13}C_{control}) / \Delta^{13}C; \quad (2)$$

where $\delta^{13}C_{soil+litter}$ is the $\delta^{13}C$ of the C fluxes and pools in the 'soil + litter' treatments, $\delta^{13}C_{control}$ is the corresponding ^{13}C signature measured in the adjacent 'bare soil' plot and $\Delta^{13}C$ is the difference in the $\delta^{13}C$ between the bulk litter (–38.4 and –40.8‰.) and the SOC (–26.7 to –27.8‰).

The seasonal and annual DOC fluxes from the litter layer and in the mineral soil were calculated by multiplying the measured DOC concentrations with the vertical water fluxes simulated with the COUP model (Jansson & Karlberg, 2001). To estimate the cumulative release of CO_2 from the litter, I developed a new model which accounts for both the temperature dependency of the C mineralisation and the declining decomposability of litter C (details in **Paper I** and **II**).

In collaboration with the EMPA, I had the opportunity to employ a QCL-based spectrometer to measure the daily variations in $\delta^{13}C_{resp}$ and $\delta^{18}O_{resp}$ in the different litter treatments (**Paper III**). This additional field campaign was conducted five months after the litter addition from April 18–28, 2008. The QCLS was coupled with a closed soil-chamber system and analysed the concentration and the isotopic composition of CO_2 at every second for 20 min of CO_2 accumulation. The soil chambers were moved manually between the different treatments throughout day and night.

3.3 Different approaches to estimate the $\delta^{13}C$ of soil-respired CO_2

As mentioned in chapter 1.3, the determination of the stable isotope ratios of soil-respired CO_2 is complicated through the dilution of soil-born CO_2 with atmospheric CO_2. In my thesis, I used three different approaches to estimate $\delta^{13}C_{resp}$: Keeling plots, two end-member mixing models and flux ratios. All three approaches have in common that they are based on the CO_2 accumulation in closed soil chambers.

(1) Keeling plot approach: This method first described by Keeling (1958) is commonly used to estimate the isotopic signature of ecosystem respiration (Pataki et al., 2003), but it has also been applied in soil-respiration studies (e.g. Ngao et al., 2005; Joos et al., 2008). Here, several gas samples are taken from a closed soil chamber with a defined temporal interval and analysed for both the CO_2 concentration and the isotopic signature. The value for the $\delta^{13}C_{resp}$ and $\delta^{18}O_{resp}$ is then given by the intercept of a simple linear regression through the plot of the stable isotope ratios and the reciprocal values of the CO_2 concentrations. I used this so-called Keeling plots to analyse the data provided by the QCL-based spectrometer (**Paper III**).

(2) Two end-member mixing model: I applied this method for the IRMS measurements (**Paper I**, **II**) because, in contrast to Keeling plots, it requires only two gas samples to estimate $\delta^{13}C_{resp}$, and thus allows more samples to cover the spatial and temporal variability in $\delta^{13}C_{resp}$. The first gas sample was collected next to each litter plot immediately after the soil chamber was closed (ambient CO_2), and the second one was taken from the chamber after a certain time of CO_2 accumulation (8–40 min). The $\delta^{13}C_{resp}$ was then calculated as:

$$\delta^{13}C_{resp} = (\delta^{13}C_{chamber} \times [CO_2]_{chamber} - \delta^{13}C_{ambient} \times [CO_2]_{ambient}) / ([CO_2]_{chamber} - [CO_2]_{ambient}), \quad (3)$$

where $[CO_2]$ is the concentration and $\delta^{13}C$ the isotopic composition of CO_2 in the ambient air and in the soil chamber. In fact, this mixing model which has been used in many soil respiration studies (e.g. Subke et al., 2004; Sakata et al., 2007; Rubino et al., 2010) corresponds with a two-point Keeling plot. The robustness of this approach is demonstrated by Steinman et al. (2004). The small number of data points only slightly reduces the precision of the estimates for $\delta^{13}C_{resp}$ because the uncertainty of the intercept extrapolation primarily depends on the range in the CO_2 concentration (Pataki et al., 2003). In a previous test, I found indeed that the values of $\delta^{13}C_{resp}$ estimated from either two or five-point Keeling plots differed only +/-0.39‰ when the range in the CO_2 concentration was the same.

Flux-ratio method: An interesting alternative to Keeling plots is the determination of $\delta^{13}C_{resp}$ directly from the flux ratio of $^{12}CO_2$ and $^{13}CO_2$:

$$\delta^{13}C_{resp} = \left(\frac{F^{13}CO_2 / F^{12}CO_2}{R_{VPDB}} - 1 \right) \times 1000‰ \quad (4)$$

In this equation, $F^{13}CO_2$ and $F^{12}CO_2$ are the soil effluxes of both isotopologues and R_{VPDB} is the standard molar ratio of ^{12}C and ^{13}C. The flux-ratio method has already been used to estimate $\delta^{13}C_{resp}$ from isotopic gradients over agricultural field sites (Griffis et al., 2004; Drewitt et al., 2009), but, to my knowledge, it has never been applied in soil chamber studies. In **Paper III**, the flux ratios are compared with the Keeling plot intercepts. The high temporal resolution of the QCLS measurements enabled to calculate the effluxes of $^{12}CO_2$ and $^{13}CO_2$ both with simple linear regressions and with second-order polynomial functions.

4. Results and Discussion

4.1 Recent litter is a minor source of microbial C and DOC in the mineral soil

The tracking of ^{13}C-labelled litter provided a deep insight into the C cycling in forest soils in the Swiss Jura mountains. **Paper I** of this thesis shows, for instance, that the added litter was only a minor source of microbial C in the top soil. The fraction of litter-derived C in the microbial biomass at 0–2 cm depth ranged from 3 to 9% at all three sampling dates (4, 8, 12 months after litter addition). Hence, the microbes consumed primarily older SOM. This finding confirms results from a recent ^{14}C-tracer study on the Oak Ridge Reservation, where in top soils less than 10% of the microbial C originated from 1–4 year old litter (Kramer et al., 2010).

Recent litter C contributed also little to the DOC fluxes in the mineral soil (**Paper I** and **II**). The 'new' C accounted, on average, for only 13% of the DOC leached at a depth of 5 cm (Fig. 3). These small amounts of litter DOC (0.6–1.8 g C m^{-2}) corresponded to less than 10% of the DOC leached from the litter layer. Thus, most litter-derived DOC was retained in the top centimetres of the mineral soil and the DOC leached below 5 cm originated mainly from the mineral soil itself. These results are in line with recent ^{13}C- and ^{14}C-tracer studies, which have found strong retentions of litter DOC for both mineral soils (Fröberg et al., 2009) and organic layers (Fröberg et al., 2007; Müller et al., 2009).

As discussed in **Paper I** and **II**, both physico-chemical sorption to mineral surfaces and biodegradation might have contributed to the removal of litter DOC in the mineral soil. For instance, litter-derived DOC in the more acidic Cambisol was retained more effectively than in the Rendzina possibly due to a stronger sorption to soil minerals at lower pH values (Tipping, 2002). After four winter months, on the other hand, the microbial biomass extracted from the mineral soil contained small but significant amounts of litter-derived C. This C probably originated from litter DOC as the cold temperatures might have prevented the transport of litter material by invertebrates. A rough mass balance estimated that 20–40% of the litter DOC could have been biologically immobilized (**Paper I**).

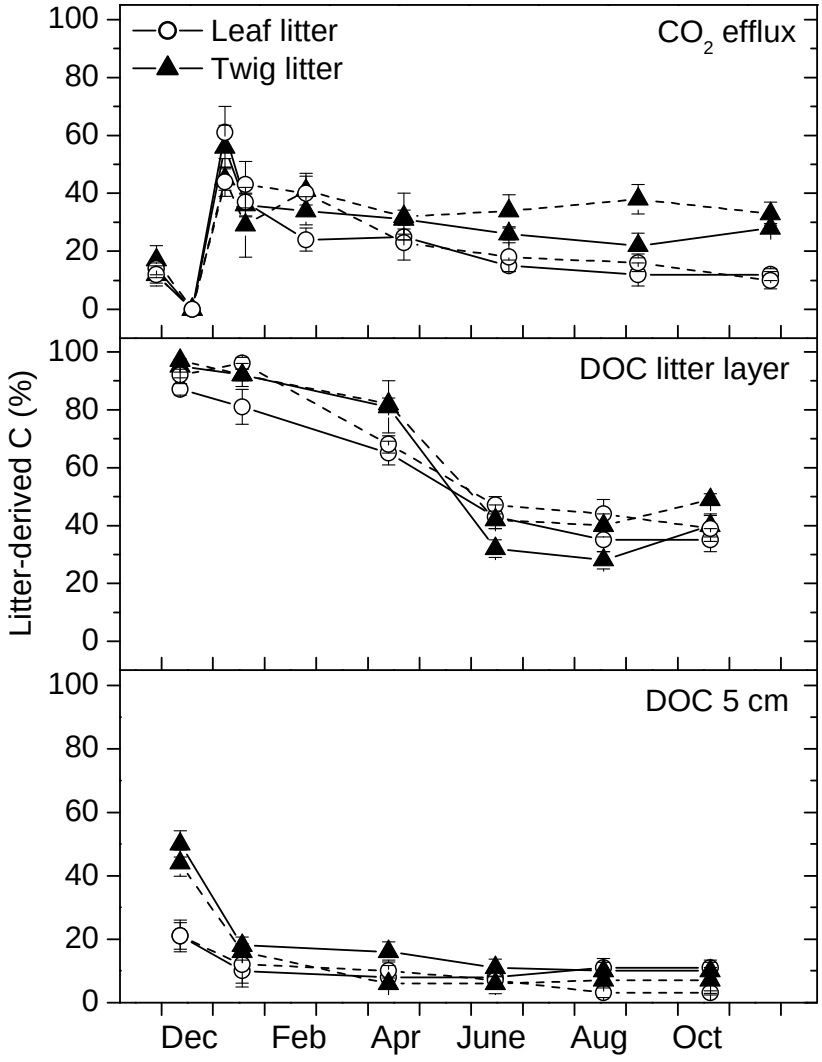

Figure 3. *Contribution of litter-derived C to the heterotrophic soil respiration and to the DOC leached from the litter layer and from the mineral soil at a depth of 5 cm. Means and standard errors of five replicates in the Rendzina (solid line) and the Cambisol (dashed line).*

Interestingly, non-litter C contributed substantially to the microbial C and the DOC not only in the mineral soil, but also in the litter layer. The ^{13}C labelling revealed that the microbial biomass contained 10–20% of unlabelled C and the DOC leached from the litter layer up to 60% after the green up of trees in spring (Fig. 3, **Paper I**). This indicates an input of non-litter C to the litter layer of more than 15 g m^{-2} yr^{-1}, which probably originated from both throughfall DOC and the deposition of pollen and other particulate organic matter.

4.2 Almost equal mineralisation of ^{13}C-labelled leaf and twig litter

Fine-woody litter is commonly thought to decompose much more slowly than leaf litter (Liski et al., 2005). One year after litter addition, the fraction of C that remained on the soil surface was indeed more than twice as much for twig litter (60%) as it was for leaf litter (23–30%; Fig. 4). My results show, however, that microbial decomposition was not the main reason for the different mass losses from the ^{13}C-labelled leaves and twigs in the forest floor. Contrary to my expectations, the mineralisation of the leaf and twig litter differed surprisingly little. Cumulated over one year, the leaves had lost 29–34% of their initial C through CO_2 and the twigs 22–26% (Fig. 4 and 5; **Paper II**). These results are supported by the measurements with the QCL-based spectrometer five months after the litter addition (**Paper III**). For two days of non-stop measurements, the twig-derived C was mineralised only 20% less rapidly than

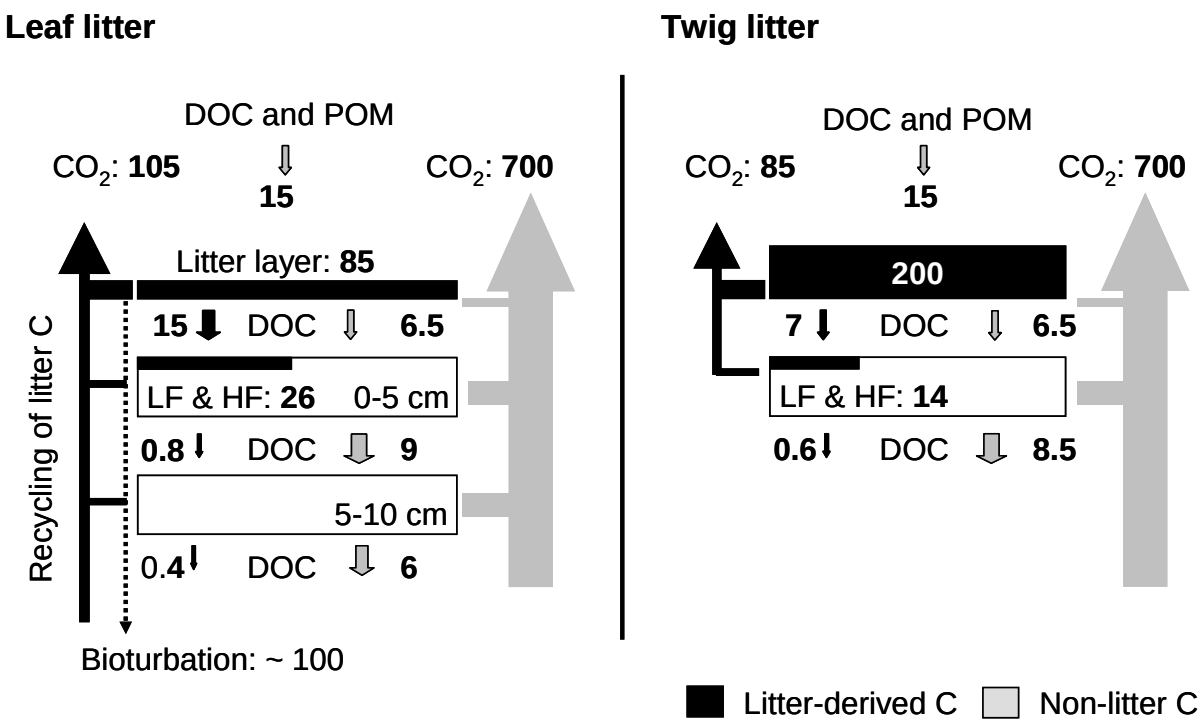

Figure 4. *Cumulated C fluxes (g C m^{-2}) from the ^{13}C-labelled leaf and twig litter and from non-litter C (e.g. 'old' SOC) over the course of one year (November 07–November 08). In addition, the litter-derived C recovered in the litter layer as well as in the light (LF; < 1.6 g cm^{-3}) and the heavy fraction (HF) at the end of the experiment is shown. The litter transport by bioturbation was not directly measured but estimated from the gap in the ^{13}C-mass balance. All values are means of ten litter plots (Rendzina + Cambisol) and are related to an annual litter input of 345 g C m^{-2} (~ 750 g dry matter).*

the leaf-derived C. In addition, twig litter placed in litterbags of 1 mm mesh-size lost only slightly less C than leaf litter confined in litterbags (30.5% vs. 32.5%; p = 0.19; **Paper II**). In comparison, litterbag studies in China and along a climatic gradient in Finland found that leaf and needle litter from *Tilia*, *Betula*, *Picea* and *Pinus* lost about twice as much C than the twig litter (Zhongling et al., 2007; Vávřová et al., 2009).

As discussed in **Paper II,** similar decay rates for fine-woody and non-woody litter might be a particular phenomenon for beech forests since beech leaves have proved to be more resistant against microbial decay than most other leaf litter (Moore et al., 1999; Hagedorn & Machwitz, 2007). Beech leaves are known to have comparatively high concentrations of lignin and polyphenols and small proportions of water solubles (Hagedorn & Machwitz, 2007; Schaefer et al., 2009). My results are supported by the mass losses from litterbags (mesh-sizes of 0.02–2 mm) on a Rendzina soil near Basel (Switzerland), which were very similar for beech leaves and spruce branchlets after one year of decomposition (Hättenschwiler et al., 1999). Moreover, almost identical rates of C mineralisation for both litter types were found in a lab experiment using a mixture of beech and oak litter (Park et al., 2002).

4.3 Incorporation of litter-derived C into mineral soils

The fact that after one year much more leaf litter C had disappeared from the litter layer (~ 70%) than had been respired in form of CO_2 (~ 30%; Fig. 4) suggests that substantial amounts of the leaf-derived C were incorporated into mineral soils (~ 40%). However, the C from the ^{13}C-labelled litter that was recovered in the first centimetres of the mineral soil was only about 4% of the initial leaf C in the light fraction (LF) and 4% in the heavy fraction (HF) (**Paper I**). Thus, there is a gap in the ^{13}C-mass balance of about 30%, which can probably be attributed to a biologically mediated transport of the leaf litter to deeper soil layers, where it was no longer detectable as the ^{13}C label vanished in the large SOC pool.

My thesis suggests that in beech forests with mull-type organic layers, bioturbation is the dominant transport pathway of leaf litter C into mineral soils, while leaching seems to be less important than in coniferous ecosystems with thick organic layers (e.g. Neff & Asner, 2001; Hagedorn et al., 2008; Kalbitz & Kaiser, 2008). For one year, the leaf litter lost 'only' 4–5% of its initial C pool through DOC leaching, corresponding to 11–16% of the litter C respired as CO_2, whereas the net export via soil fauna (mainly invertebrates) might have amounted to more than 30% of the initial leaf litter C.

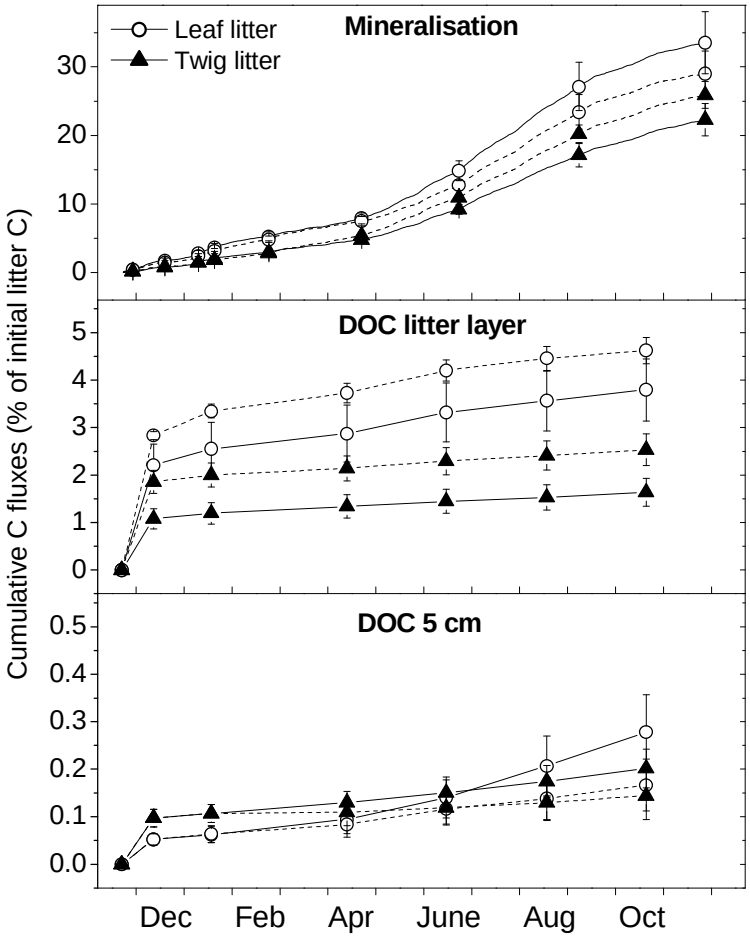

Figure 5. *Seasonal dynamic of litter-derived C respired as CO_2, leached as DOC from the litter layer and recovered in the DOC at a depth of 5 cm. The solid line represents the Rendzina and the dashed line the Cambisol. All values are the means of five replicates (± standard error).*

As shown in **Paper II**, twig-derived C was transported downwards much more slowly than leaf-derived C by both DOC leaching and bioturbation (Fig. 4): (1) Leaching rates of DOC from the twigs amounted only to half of that from the leaves throughout the experiment (Fig. 5). One reason why the DOC leaching differed much more between leaf and twig litter than the C mineralisation was possibly the limited contact of the inner parts of the twigs with percolating water. (2) At the end of the experiment, less of the initial twig C than of the leaf C was recovered at 0–2 cm depth (4% vs. 8%). (3) The gap in the ^{13}C-mass balance was much smaller for the twig than for the leaf litter. By summing up across all C fluxes and pools that had been measured, about 90% of the added twig litter C was recovered. Consequently, only a small amount of the twig-derived C was translocated to soil depths below 2 cm.

In summary, the present thesis shows that freshly fallen beech leaves are rapidly incorporated into mineral soils, while fine-woody litter decomposes largely *in situ* on the soil surface. Twig litter will probably not be transported downwards until twigs lose their rigid structure and break down into smaller pieces. By that stage of decomposition, however, a large proportion of the twig-derived C might have already been mineralised to CO_2, and thus would not contribute to C storage in mineral soils. This finding as well as the fact that fine-woody litter accounts for 'only' 30% of the annual litter fall (leaf litter 70%) suggests that decomposing twigs are clearly less important for the C storage in these soils than leaves.

4.4 Litter decomposition in winter

Although in deciduous forests, most litter falls in autumn, little is known about the fate of this fresh litter C over the winter months. Is it preserved due to the cold temperatures or partly mineralised due to its high decomposability? **Paper I** suggests that during winter, primarily the most labile litter compounds such as hydrophilic substances were decomposed, whereas more recalcitrant components were largely preserved. After five winter month, the leaf litter had lost only about 11% of its initial C and the twig litter 7%. Approximately 30% of this C loss can be attributed to an initial DOC flush of water soluble substances (**Paper I**), which contributed to 80% of the annual leaching losses (Fig. 5). In contrast, respiration during the cold season accounted for only 20–25% of the annual C losses from the litter through CO_2. The seasonal dynamic of litter-derived CO_2 and DOC fluxes differed only slightly between leaf and twig litter (Fig. 5; **Paper II**), possibly because both litter types contained a relatively small proportion of water soluble substances and were rich in refractory components (lignin, tannin etc.).

4.5 Contribution of litter-derived C to soil CO_2 effluxes (f_{litter})

One aim of this thesis was to assess how much the decomposition of recent litter (< 1 yr) contributes to soil CO_2 effluxes at the Lägeren research site. **Paper I** and **II** show that litter-derived CO_2 can indeed be a major component of soil CO_2 effluxes (45–60%), particularly on warm winter days when the leaf litter is still fresh (Fig. 3). From November to December, however, a very cold (0–1°C) or frozen litter layer on soils with temperatures above 3°C only contributed negligibly to soil CO_2 effluxes despite the very fresh litter C. Over the warm season, the variability in f_{litter} was much less pronounced than in winter and the maximum values for f_{litter} were on a clearly lower level (Fig. 3). Here, it should be noted that the litter

layer was mostly wet throughout summer 2008 with frequent rain. Drying and wetting cycles may result in large fluctuations of f_{litter} especially during the warm season (Cisneros-Dozal et al., 2006). By combining the C mineralisation rates (22–34% C loss yr^{-1}) with the amounts of litterfall, I estimated that the decomposition of recent leaf litter contributed to 10–12% of the annual C losses from soils and recent twig litter 4–6% (**Paper II**)

4.6 Application of a quantum cascade laser-based spectrometer

My thesis presents the first application of a quantum cascade laser-based spectrometer (QCLS) in a closed soil-chamber system to analyse the $\delta^{13}C$ and $\delta^{18}O$ of soil-respired CO_2 ($\delta^{13}C_{resp}$, $\delta^{18}O_{resp}$). On two days in early spring, 90 chamber measurements with 20 min sampling time each were performed in all litter treatments. The QCLS measured the $\delta^{13}C$ and $\delta^{18}O$ of the CO_2 that accumulated in the chamber headspace with a one-second interval and a precision of 0.25‰, resulting in Keeling plots of 1200 data points (Fig. 6). **Paper III** shows that this new method can be used to reliably estimate $\delta^{13}C_{resp}$ and $\delta^{18}O_{resp}$. This requires, however, a thorough assessment of the huge data set produced because the isotopic effluxes of CO_2 were significantly affected through the soil-chamber system.

Most surprisingly, the $\delta^{13}C$ of the accumulating CO_2 started to oscillate (up to ±1.5‰) in the second part of each measurement (Fig. 6). The reason for these oscillations remains unclear; possibly they were caused by a stratification of CO_2 with different ^{13}C ratios in the chamber headspace due to insufficient mixing of the chamber air. However, they cannot be explained either by an instrumental instability or a leak in the chamber system because we found no fluctuations in the gas temperature, the pressure, the laser intensity or in the $^{12}C^{16}O_2$ and $^{12}C^{16}O^{18}O$ signal. Also pressure pumping – for instance through wind – could be rule out. The use of ‘moving windows’ of 400 data points revealed a systematic shift in $\delta^{13}C_{resp}$ of on average +1.9‰ in the second part of the measurements (Fig. 7). This bias might indicate a so-called 'diffusive-kinetic fractionation’ as it has recently been described by Nickerson & Risk (2009a). They show that, through chamber-to-soil feedbacks, the flux rate of the faster-diffusing $^{12}CO_2$ is reduced in comparison to that of $^{13}CO_2$, leading to significant concave-up curvature of the Keeling plots. While their results are mainly based on different simulation scenarios, **Paper III** has proved this phenomenon, to my knowledge, for the first time in a field experiment. In ordinary Keeling plot studies using IRMS such a bias is probably difficult to recognize because only a limited number of gas samples is used (e.g. Flanagan et al., 1999; Ngao et al., 2005).

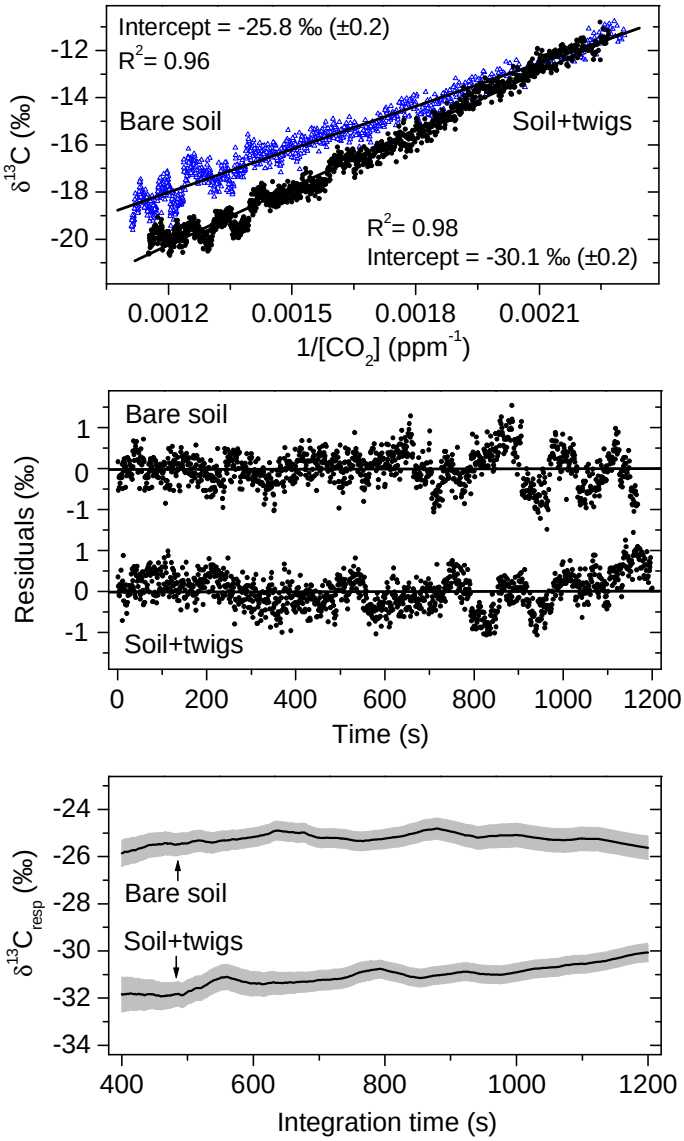

Figure 6. *$\delta^{13}C$-Keeling plots (upper figures) and residuals of the least square fits (middle figures) for two typical 20-min measurements, one with a pronounced variability in $\delta^{13}C_{resp}$ ('soil+twigs') and the other with a small variability ('bare soil'). The figures below show changes in estimates of $\delta^{13}C_{resp}$ when an increasing number of data points is used for linear regression (from the first 400 s to the entire Keeling plot). The grey band represents the 95%-confidence interval of $\delta^{13}C_{resp}$.*

The $\delta^{18}O$-Keeling plots showed no systematic fluctuations in the $\delta^{18}O$ values. However, they were much more bended than the $\delta^{13}C$-Keeling plots probably due to the invasion of chamber CO_2 into the first few centimetres of soil, where the ^{18}O of CO_2 equilibrated partly with the soil water before it diffused back to the headspace. This feedback effect has been discussed in other studies (Tans, 1998; Miller et al., 1999), and has also been observed in static closed soil chambers (Mortazavi et al., 2004). Our high resolution Keeling plots, however, provide unique insights into the dynamic of this process, and also allowed for a new method to estimate $\delta^{18}O_{resp}$ (for details see **Paper III**).

I assume that the soil-chamber feedback demonstrated in **Paper III** also slightly biased the estimates of $\delta^{13}C_{resp}$ used in **Paper I** and **II** to calculate the litter contribution to soil CO_2 effluxes (f_{litter}). Averaged across all samplings (November 07–November 08), the $\delta^{13}C_{resp}$ in the bare-soil treatment was increased by 1.5‰ relative to the $\delta^{13}C$ of the SOM at 0–10 cm

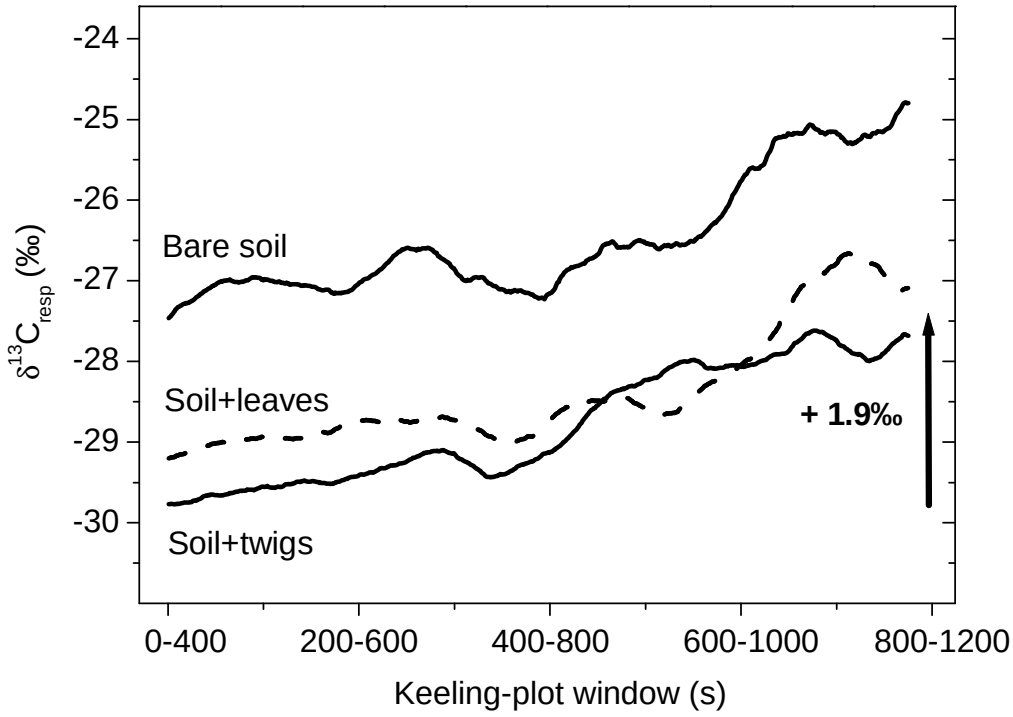

Figure 7. *Temporal course of $\delta^{13}C_{resp}$ for 20 min of CO_2 accumulation, estimated with 'moving windows' of 400 data points. The curves represent mean values for the different litter treatments (bare soil: n = 12; soil + leaves: n = 30; soil + twigs: n = 31).*

depth in both soils (**Paper II**). This positive shift in $\delta^{13}C_{resp}$ is in conflict with the ^{13}C-mass balance, since several lab incubations have shown that isotopic fractionation during decomposition of litter and SOM is either negligible or leads to a slight ^{13}C depletion of the respired CO_2 as compared to its source (Schweizer et al., 1999; Santruckova et al., 2000; Rubino et al., 2009). My results support these studies as the $\delta^{13}C$ of the litter material did not change significantly for one year of decomposition (**Paper II**). In a recent review, Bowling et at. (2008) show that gaps in the ^{13}C-mass balance can be found in most soil-respiration studies. They argue that the increased values of $\delta^{13}C_{resp}$ are caused by soil-chamber feedbacks.

Nevertheless, I feel confident that in my tracer experiment such an overestimation of $\delta^{13}C_{resp}$ might have biased the values of f_{litter} only marginally. **Paper III** shows that the bias in $\delta^{13}C_{resp}$ was identical for all litter treatments in case of equal chamber-closer times (see also Fig. 7). Furthermore, I found only small differences between f_{litter} estimated from IRMS measurements (Eq. 3) one week before the laser experiment and f_{litter} derived from the high-resolution Keeling plots, in which the bias in $\delta^{13}C_{resp}$ was taken into account.

4.7 Improving the estimates of $\delta^{13}C_{resp}$

In contrast to most soil-chamber studies using IRMS, the data set from the QCLS allowed us to detect non-linearities in the δ^{13}C-Keeling plots. This information helped us to improve the estimates of $\delta^{13}C_{resp}$. For instance, we used only the first 10 out of 20 min of CO_2 accumulation, for which no systematic error in $\delta^{13}C_{resp}$ was observed. In addition, we tested whether the flux-ratio method (Eq. 4) can be used alternatively to Keeling plots to calculate $\delta^{13}C_{resp}$. **Paper III** demonstrates that this approach may account better for chamber feedbacks on $\delta^{13}C_{resp}$ than the Keeling approach, but only when the isotopic flux rates are derived from quadratic fits. These estimates are, however, less robust than Keeling plots, and thus only suitable when adequate numbers of replicates of the same treatment are used.

Even though we significantly improved the estimates of $\delta^{13}C_{resp}$ by post processing, there still might be uncertainties exceeding ±1‰. Over two days, the range in $\delta^{13}C_{resp}$ measured in the same treatment was 3–5.5‰ (**Paper III**). This variability could have been caused by physical factors leading to advection and diffusion of isotopically enriched soil gas (Maseyk et al., 2009; Nickerson & Risk, 2009c; Kayler et al., 2010). However, this assumption cannot be proved as there were no significant correlations between $\delta^{13}C_{resp}$ and physical variables (air temperature, ambient CO_2 concentration, air pressure, wind etc.). Similar short-term variations in $\delta^{13}C_{resp}$ have recently been observed in trenched soils under deciduous trees using a TDL spectrometer coupled with an open chamber system (Moyes et al., 2010). For the partitioning of soil respiration with small ^{13}C labels, such variability requires that $\delta^{13}C_{resp}$ in control and treatment plots is measured within short time, or even simultaneously, and with several replicates as this was implemented in the sampling design of my ^{13}C-tracer experiment (**Paper I** and **II**).

5. Conclusions

The results of my thesis contribute the following novel information to our present knowledge on the decomposition of litter and to the improvement of stable isotope analyses in soil-respiration studies:

- The C mineralisation of woody tissue must not basically be slow, as assumed in most soil C models. Over one year, the ^{13}C-labelled twig litter lost only 10–35% less C through mineralisation than the ^{13}C-labelled leaf litter. This small difference seems to be a particular phenomenon of beech forests.
- Decaying twigs might be clearly less important for the C storage in mineral soils than leaf litter. While about 40% of the leaf-derived C was incorporated into the mineral soil, the twig litter mainly decomposed *in situ* on the soil surface due to both a small leaching of DOC and a slow downward transport via soil fauna.
- The greatest contribution of recent litter to the soil respiration can be expected on warm winter days (> 50%) when the mineral soil is still cold and the labile litter pool is only partly mineralised. Nevertheless, the most prominent process of litter decomposition during the cold season is probably the leaching of DOC, accounting for about 80% of the annual DOC losses from litter, whereas the litter mineralisation in winter contributed to only 20–25% of the annual C losses from the litter through CO_2.
- The combination of QCL-based spectrometers with closed soil chambers is a promising approach to reliably estimate $\delta^{13}C_{resp}$ and $\delta^{18}O_{resp}$. The high temporal resolution of QCLS measurements allows the detection of non-linearities in the isotopic effluxes of CO_2 from the soil due to soil-chamber feedbacks. This information can be used to improve the estimates of $\delta^{13}C_{resp}$ and $\delta^{18}O_{resp}$.

Furthermore, my results confirm recent findings and assumptions that:

- Beech leaves are mineralised relatively slowly because they are rich in refractory components (lignin, tannin etc.), but comparatively poor in water soluble substances.
- Litter-derived CO_2 is a highly variable component of the soil respiration.
- Most of the DOC leached from the forest floor is retained in the top centimetres of the mineral soil through both physico-chemical sorption to mineral surfaces and biodegradation. Thus, DOC from freshly fallen litter (< 1 yr) contributes only slightly to the DOC fluxes in the top soil (< 10%).
- Recent litter C is only a minor source of microbial C in the mineral soil.
- During the CO_2 accumulation in soil chambers, the isotopic composition of soil CO_2 effluxes is affected significantly by soil-chamber feedbacks.
- The ^{13}C and ^{18}O signature of the heterotrophic soil respiration can greatly vary for short time periods (hours) without following a daily cycle.

6. Research Perspectives

This thesis demonstrates the power of stable isotopes in investigating the pathways of decomposing litter. Moreover, it presents new approaches to improve the application of this technique in soil-respiration studies. In future studies on litter decomposition, therefore, I would recommend to take advantage of isotopically labelled litter when-ever possible. Given the small number of tracer studies to date (Table 1), there is still a lack of knowledge about the fate of litter-derived C in many forest ecosystems and of many tree species.

My tracer experiment shows that beech twigs are rapidly mineralised, but might be less important for the C storage in forest soils as previously thought. It would be interesting to see whether this pattern can be extrapolated to other beech forest ecosystems or even to twig litter from other tree species. Furthermore, it would be desirable to run tracer studies for longer time periods (> 1 yr) to investigate the pathways of the added litter also in latter stages of decomposition. However, this requires an isotopic label which is much stronger than that used in my tracer experiment. Highly labelled litter may also allow the litter-derived C to be followed into deeper layers of the mineral soil (Bird & Torn, 2006; Rubino et al., 2010). But unfortunately, the production of such litter is relatively time and cost intensive particularly for woody litter. By contrast, the numerous free air CO_2-enrichment experiments (FACE) performed for the last two decades have yielded large amounts of slightly labelled litter as a by-product, which can now be used in litter experiments.

After one year of decomposition, the ^{13}C-labelled litter accounted for small but significant fractions of the C in the light (6%) and the heavy fraction (3%) as well as in the microbial biomass (5%) at 0–2 cm depth. However, my results do not show which components of the litter (e.g. sugars, lignin) contributed preferentially to this new C recovered in the mineral soil. Moreover, the chloroform-fumigation method used to determine the microbial biomass allows no information on which parts of the microbial community (e.g. bacteria vs. fungi) participated in the mineralisation and transformation process of litter-derived C. Here, a more powerful approach is the analysis of the ^{13}C signature in microbial phospholipids fatty acids (Kramer et al., 2010; Rubino et al., 2010). Such investigations on a molecular level could

help to identify the main drivers of C storage in forest soils and will provide additional insights into the mechanistic of litter decomposition.

Another focus of future research should be to further improve the determination of soil CO_2 effluxes and especially their isotopic composition. It is important to assess whether the soil-chamber feedbacks on $\delta^{13}C_{resp}$ and $\delta^{18}O_{resp}$ as found in this thesis and other recent studies (e.g. Nickerson & Risk, 2009b) are a source of error in most respirations studies where soil chambers are employed. For these investigations, I suggest combined approaches based on both modelling of the CO_2 diffusion through the soil porous system and analyses of the CO_2 accumulation in soil chambers with a high temporal resolution by laser spectroscopy. Future studies in this field should also thoroughly test whether closed or open chamber systems are more suitable to estimate $\delta^{13}C_{resp}$ and $\delta^{18}O_{resp}$.

The application of laser spectrometry in litter experiments has the potential to make an important step forward in our understanding of litter decomposition. As an example, it could be used to investigate how drying-wetting and freezing-thawing cycles affect the respiration of C from ^{13}C-labelled litter in the field. The analysis of this short-term dynamics requires measurements with a high temporal resolution. In **Paper I** and **II** of this thesis, I present a new model approach to estimate the cumulative C losses from litter through CO_2 from a small number of sampling dates. This model could be clearly improved by using continuous measurements of the CO_2 efflux from litter.

So far, both the development and the parameterisation of most soil C models have been largely based on the knowledge from litterbag and lab-incubation studies. However, if the number of tracer studies will further increase, which is very likely, it seems to be a great opportunity to include their results in more complex C models. These models may better account for the different pathways of decomposing litter, and thus may allow better predictions of C stocks in soils under a changing climate and land-use.

List of References

Bahn M., Schmitt M., Siegwolf R., Richter A., Brüggemann N. (2009) Does photosynthesis affect grassland soil-respired CO_2 and its carbon isotope composition on a diurnal timescale?. New Phytologist, 182, 451–460.

Bird J. A., Kleber M., Torn M. S. (2008) ^{13}C and ^{15}N stabilization dynamics in soil organic matter fractions during needle and fine root decomposition. Organic Geochemistry, 39, 465–477.

Bird J. A., Torn M. S. (2006) Fine roots vs. needles: a comparison of ^{13}C and ^{15}N dynamics in a ponderosa pine forest soil. Biogeochemistry, 79, 361–382, doi:10.1007/s10533-005-5632-y.

Borken W., Davidson E. A., Savage K., Gaudinski J., Trumbore S. E. (2003) Drying and wetting effects on carbon dioxide release from organic horizons. Soil Science Society of America Journal, 67, 1888–1896, doi: 10.2136/sssaj2003.1888.

Bowling D. R., Sargent S. D., Tanner B. D., Ehleringer J. (2003) Tunable diode laser absorption spectroscopy for stable isotope studies of ecosystem–atmosphere CO_2 exchange. Agricultural and Forest Meteorology, 118, 1–19.

Bowling D. R., Pataki D. E., Randerson J. T. (2008) Carbon isotopes in terrestrial ecosystem pools and CO_2 fluxes. New Phytologist, 178, 24–40.

Cisneros-Dozal L. M., Trumbore S., Hanson P. J. (2006) Partitioning sources of soil-respired CO_2 and their seasonal variation using a unique radiocarbon tracer. Global Change Biology, 12, 194–204, doi: 10.1111/j.1365-2486.2005.01061.x.

Cotrufo M. F., Ngao J., Marzaioli F., Piermatteo D. (2010) Inter-comparison of methods for quantifying above-ground leaf litter decomposition rates. Plant Soil, 334, 365–376, doi:10.1007/s11104-010-0388-0.

Crow S. E., Lajtha K., Filley T. R., Swanston C. W., Bowden R. D., Caldwell B. A. (2009) Sources of plant-derived carbon and stability of organic matter in soil: implications for global change. Global Change Biology, 15, 2003–2019, doi: 10.1111/j.1365-2486.2009.01850.x.

Dawson T. E., Mambelli S., Plamboeck A. H., Templer P. H., Tu K. P. (2002) Stable isotopes in plant ecology. Annual Review of Ecological Systems, 33, 507–559.

Drewitt G., Wagner-Riddle C., Warland J. (2009) Isotopic CO_2 measurements of soil respiration over conventional and no-till plots in fall and spring. Agricultural and Forest Meteorology, 149, 614–622, doi: 10.1016/j.agrformet.2008.10.009.

Flanagan L. B., Kubien D. S., Ehleringer J. R. (1999) Spatial and temporal variation in the carbon and oxygen stable isotope ratio of respired CO_2 in a boreal forest ecosystem. Tellus 51B, 367–384.

Fontaine S., Barot S., Barré P., Bdioui N., Mary B., Rumpel C. (2007) Stability of organic carbon in deep soil layers controlled by fresh carbon supply. Nature, 450, 277–280, doi:10.1038/nature06275.

Friedlingstein P., Cox P. M., Betts R., Bopp L., von Bloh W., Brovkin V., Cadule P., Doney S., Eby M., Fung I., Bala G., John J., Jones C., Joos, F., Kato T., Kawamiya M., Knorr W., Lindsay K., Matthews H. D., Raddatz T., Rayner P., Reick C., Roeckner E., Schnitzler K. G., Schnur R., Strassmann K., Weaver A. J., Yoshikawa C., Zenh N. (2006) Climate-carbon cycle feedback analysis, results from the C4MIP model intercomparison. Journal of Climate, 19, 3337–3353.

Fröberg M., Berggren Kleja D., Hagedorn F. (2007) The contribution of fresh litter to dissolved organic carbon leached from a coniferous forest floor. European Journal of Soil Science, 58, 108–114, doi:10.1111/j.1365-2389.2006.00812.x.

Fröberg M., Hanson P. J., Trumbore S. E., Swanston C. W., Todd D. E. (2009) Flux of carbon from ^{14}C-enriched leaf litter throughout a forest soil mesocosm. Geoderma, 149, 181–188, doi:10.1016/j.geoderma.2008.11.029.

Goodale C. L., Apps M. J., Birdsey R. A., Field C. B., Heath L. S., Houghton R. A., Jenkins J. C., Kohlmaier G. H., Kurz W., Liu S., Nabuurs G. J., Nilsson S., Shvidenko A. Z. (2002) Forest carbon sinks in the northern hemisphere. Ecological Applications, 12, 891–899.

Griffis T. J., Baker J. M., Sargent S. D., Tanner B. D., Zhang J. (2004) Measuring field-scale isotopic CO_2 fluxes with tunable diode laser absorption spectroscopy and micrometeorological techniques. Agricultural and Forest Meteorology, 124, 15–29, doi:10.1016/j.agrformet.2004.01.009.

Guo Z., Li Q., Zheng J., Liu W., Fan C., Ma Y., Yu G., Han S. (2007) Leaf and twig litter decomposition of main species in different forests along the north slope of Changbai Mountain, northeast China. Frontiers of Forestry in China, 2, 47–54, doi 10.1007/s11461-007-0007-2.

Hagedorn F., Machwitz M. (2007) Controls on dissolved organic matter leaching from forest litter grown under elevated atmospheric CO_2. Soil Biology and Biochemistry, 39, 1759–1769, doi:10.1016/j.soilbio.2007.01.038.

Hagedorn F., Maurer S., Bucher J. B., Siegwolf R. T. W. (2005) Immobilization, stabilization and remobilization of nitrogen in forest soils at elevated CO_2: a ^{15}N and ^{13}C tracer study. Global Change Biology, 11, 1816–1827, doi:10.1111/j.1365-2486.2005.01041.x.

Hagedorn F., van Hees P. A. W., Handa I. T., Hättenschwiler S. (2008) Elevated atmospheric CO_2 fuels leaching of old dissolved organic matter at the alpine treeline. Global Biogeochemical Cycles, 22, GB2004, doi:10.1029/2007GB003026.

Hättenschwiler S., Bühler S., Körner C. (1999) Quality, decomposition and isopod consumption of tree litter produced under elevated CO_2. Oikos, 85, 271–281.

Heim A., Wehrli L., Eugster W., Schmidt M. W. I. (2009) Effects of sampling design on the probability to detect soil carbon stock changes at the Swiss CarboEurope site Lägeren. Geoderma, 149 (3–4), 347–354.

IPCC (2007) Climate Change 2007: The physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, UK and NY, USA.

Jansson P. E., Karlberg L. (2001) Coupled heat and mass transfer model for soil-plant-atmosphere systems. ftp://www.lwr.kth.se/CoupModel/CoupModel.pdf. Accessed 22 September 2009.

Joos O., Hagedorn F., Heim A., Gilgen A. K., Schmidt M. W. I., Siegwolf R. T. W., Buchmann N. (2010) Summer drought reduces total and litter-derived soil CO_2 effluxes in temperate grassland – clues from a ^{13}C litter addition experiment. Biogeosciences, 7, 1031–1041, doi:10.5194/bg-7-1031-2010.

Joos O., Saurer M., Heim A., Hagedorn F., Schmidt M. W. I., Siegwolf, R. T. W. (2008) Can we use the CO_2 concentrations determined by continuous-flow isotope ratio mass spectrometry from small samples for the Keeling plot approach?. Rapid Communication in Mass Spectrometry, 22, 4029–4034.

Kaiser K., Guggenberger G. (2000) The role of DOM sorption to mineral surfaces in the preservation of organic matter in soils. Organic Geochemistry, 31, 711–725, doi:10.1016/S0146-6380(00)00046-2.

Kalbitz K., Kaiser K. (2008) Contribution of dissolved organic matter to carbon storage in forest mineral soils. Journal of Plant Nutrition and Soil Science, 171, 52–60, doi:10.1002/ jpln.200700043.

Kayler Z. E., Sulzman E. W., Rugh W. D., Mix A. C., Bond B. J. (2010) Characterizing the impact of diffusive and advective soil gas transport on the measurement and interpretation of the isotopic signal of soil respiration. Soil Biology and Biochemistry, 42, 435–444.

Keeling C. D. (1958) The concentrations and isotopic abundances of atmospheric carbon dioxide in rural areas. Geochimica et Cosmochimica Acta, 13, 322–334.

Kramer C., Trumbore S., Fröberg M., Cisneros Dozal L. M., Zhang D., Xu X., Santos G. M., Hanson P. J. (2010) Recent (<4 year old) leaf litter is not a major source of microbial carbon in a temperate forest mineral soil. Soil Biology and Biochemistry, 42, 1028–1037 doi:10.1016/j.soilbio.2010.02.021.

Krull E. S., Baldock J. A., Skjemstad J. O. (2003) Importance of mechanisms and processes of the stabilisation of soil organic matter for modelling carbon turnover. Functional Plant Biology, 30, 207–222.

Larocque G. R., Boutin R., Paré D., Robitaille G., Lacerte V. (2006) Assessing a new soil carbon model to simulate the effect of temperature increase on the soil carbon cycle in three eastern Canadian forest types characterized by different climatic conditions. Canadian Journal of Soil Science, 86, 187–202.

Liski J., Nissinen A., Erhard M., Taskinen O. (2003) Climatic effects on litter decomposition from arctic tundra to tropical rainforest. Global Change Biology, 9, 1–10, doi:10.1046/j.1365-2486.2003.00605.x.

Liski J., Palosuo T., Peltoniemi M., Sievänen R. (2005) Carbon and decomposition model Yasso for forest soils. Ecological Modelling, 189, 168–182, doi:10.1016/j.ecolmodel.2005.03.005.

Magill A. H., Aber J. D. (2000) Dissolved organic carbon and nitrogen relationships in forest litter as affected by nitrogen deposition. Soil Biology and Biochemistry, 32, 603–613, doi:10.1016/S0038-0717(99)00187-X.

Marron N., Plain C., Longdoz B., Epron D. (2009) Seasonal and daily time course of the ^{13}C composition in soil CO_2 efflux recorded with a tunable diode laser spectrophotometer (TDLS). Plant Soil, 318, 137–151, doi:10.1007/s11104-008-9824-9.

Maseyk K., Wingate L., Seibt U., Ghashghaie J., Bathellier C., Almeida P., Lobo de Vale R., Pereira J.S.,Yakir D., Mencuccini M. (2009) Biotic and abiotic factors affecting the $\delta^{13}C$ of soil-respired CO_2 in a Mediterranean oak woodland. Isotopes in Environmental and Health Studies, 45 (4), 343–359.

Midwood A. J., Thornton B., Millard P. (2008) Measuring the ^{13}C content of soil-respired CO_2 using a novel open chamber system. Rapid Communication in Mass Spectrometry, 22, 2073–2081.

Miller J. B., Yakir D., White J. W. C., Tans P. P. (1999) Measurement of $^{18}O/^{16}O$ in the soil-atmosphere CO_2 flux. Global Biogeochemical Cycles, 13, 761–774.

Moore T. R., Trofymow J. A., Taylor B., Prescott C., Camiré C., Duschene L., Fyles J., Kozak L., Kranabetter M., Morrison I., Siltanen M., Smith S., Titus B., Visser S., Wein R., Zoltai S. (1999) Litter decomposition rates in Canadian forests. Global Change Biology, 5, 75–82, doi:10.1046/j.1365-2486.1998.00224.x.

Moyes A. B., Gaines S. J., Siegwolf R. T. W., Bowling D. R. (2010) Diffusive fractionation complicates isotopic partitioning of autotrophic and heterotrophic sources of soil respiration. Plant, Cell and Environment, 33, 1804–1819, doi: 10.1111/j.1365-3040.2010.02185.x.

Mortazavi B., Prater J. L., Chanton J. P. (2004) A field-based method for simultaneous measurements of the $\delta^{18}O$ and $\delta^{13}C$ of soil CO_2 efflux. Biogeosciences, 1, 1–9.

Müller M., Alewell C., Hagedorn F. (2009) Effective retention of litter-derived dissolved organic carbon in organic layers. Soil Biology and Biochemistry, 41(6), 1066–1074, doi:10.1016/j.soilbio.2009.02.007.

Neff J. C., Asner G. P. (2001) Dissolved organic carbon in terrestrial ecosystems: Synthesis and a Model. Ecosystems 4, 29–48, doi:10.1007/s100210000058.

Ngao J., Epron D., Brechet C., Granier A. (2005) Estimating the contribution of leaf litter decomposition to soil CO_2 efflux in a beech forest using ^{13}C-depleted litter. Global Change Biology, 11, 1768–1776, doi: 10.1111/j.1365-2486.2004.01014.x.

Nickerson N., Risk D. (2009a) Keeling plots are non-linear in non-steady state diffusive environments. Geophysical Research Letters, 36, L08401, doi:10.1029/2008GL036945.

Nickerson N., Risk D. (2009b) A numerical evaluation of chamber methodologies used in measuring the $\delta^{13}C$ of soil respiration. Rapid Communication in Mass Spectrometry, 23, 2802–2810.

Nickerson N., Risk D. (2009c) Physical controls on the isotopic composition of soil-respired CO_2. Journal of Geophysical Research, 114, G01013, doi:10.1029/2008JG000766.

Nottingham A. T., Griffiths H., Chamberlain P. M., Stott A. W., Tanner E. V. J. (2009) Soil priming by sugar and leaf-litter substrates: A link to microbial groups. Applied Soil Ecology, 42, 183–190, doi:10.1016/j.apsoil.2009.03.003.

Ohlsson K. E. A., Bhupinderpal S., Holm S., Nordgren A., Lovdahl L., Högberg P. (2005) Uncertainties in static closed chamber measurements of the carbon isotopic ratio of soil-respired CO_2. Soil Biology and Biochemistry, 37, 2273–2276.

Park J. H., Kalbitz K., Matzner E. (2002) Resource control on the production of dissolved organic carbon and nitrogen in a deciduous forest floor. Soil Biology and Biochemistry, 34, 813–822, doi:10.1016/S0038-0717(02)00011-1.

Paustian K., Levine E., Post W. M., Ryzhova I. M. (1996) The use of models to integrate information and understanding of soil C at the regional scale. Geoderma, 79, 227–260.

Park J. H., Matzner E. (2003) Controls on the release of dissolved organic carbon and nitrogen from a deciduous forest floor investigated by manipulations of aboveground litter inputs and water flux. Biogeochemistry, 66, 265–286, doi:10.1023/B:BIOG.0000005341.19412.7b.

Pataki D. E., Ehleringer J. R., Flanagan L. B., Yakir D., Bowling D. R., Still C. J., Buchmann N., Kaplan J. O., Berry J. A. (2003) The application and interpretation of Keeling plots in terrestrial carbon cycle research. Global Biogeochemical Cycles, 17, GB1022, doi:10.1029/2001GB001850.

Pregitzer K., Euskirchen E. (2004) Carbon cycling and storage in world forests: biome patterns related to forest age. Globale Change Biology, 10, 2052–2077.

Prescott C. E. (2010) Litter decomposition: what controls it and how can we alter it to sequester more carbon in forest soils?. Biogeochemistry, 101, 133–149, doi:10.1007/s10533-010-9439-0.

Pumpanen J., Kolari P., Ilvesniemi H., Minkkinen K., Vesala T., Niinisto S., Lohila A., Larmola T., Morero M., Pihlatie M., Janssens I., Yuste J., Grunzweig J., Reth S., Subke J. A., Savage K., Kutsch W., Ostreng G., Ziegler W., Anthoni P., Lindroth A., Hari P. (2004) Comparison of different chamber techniques for measuring soil CO_2 efflux. Agricultural and Forest Meteorology, 123, 159–176, doi:10.1016/j.agrformet.2003.12.001.

Rey A., Pegoraro E., Tedeschi V., De Parri I., Jarvis P. G., Valentini R. (2002) Annual variation in soil respiration and its components in a coppice oak forest in Central Italy. Global Change Biology, 8, 851–866, doi:10.1046/j.1365-2486.2002.00521.x.

Risk D., Kellman L. (2008) Isotopic fractionation in non-equilibrium diffusive environments. Geophysical Research Letters, 35, L02403, doi:10.1029/2007GL032374.

Rubino M., Dungait J. A. J., Evershed R. P. Bertolini T., De Angelis P., D'Onofrio A., Lagomarsino A., Lubritto C., Merola A., Terrasi F., Cotrufo M. F. (2010) Carbon input belowground is the major C flux contributing to leaf litter mass loss: Evidences from a ^{13}C labelled-leaf litter experiment. Soil Biology and Biochemistry, 42, 1009–1016, doi:10.1016/j.soilbio.2010.02.018.

Rubino M., Lubritto C., D'Onofrio A., Terrasi F., Kramer C., Gleixner G., Cotrufo M. F. (2009) Isotopic evidences for microbiologically mediated and direct C input to soil compounds from three different leaf litters during their decomposition. Environmental Chemical Letters, 7, 85–95, doi: 10.1007/s10311-008-0141-6.

Ruehr N. K., Knohl A., Buchmann N. (2010) Environmental variables controlling soil respiration on diurnal, seasonal and annual time-scales in a mixed mountain forest in Switzerland. Biogeochemistry, 98, 153–170, doi:10.1007/s10533-009-9383-z.

Sakata T., Ishizuku S., Takahashi M. (2007) Separation of soil respiration into CO_2 emission sources using ^{13}C natural abundance in a deciduous broad-leaved forest in Japan. Soil Science and Plant Nutrition, 53, 328–336, doi: 10.1111/j.1747-0765.2007.00141.x.

Santruckova H., Bird M. I., Lloyd J. (2000) Microbial processes and carbon-isotope fractionation in tropical and temperate grassland soils. Functional Ecology, 14, 108–114, doi:10.1046/j.1365-2435.2000.00402.x.

Schaefer M., Migge-Kleian S., Scheu S. (2009) The role of soil fauna for decomposition of plant residues, in: Functioning and management of European beech ecosystems, edited by: Brumme R., Khanna P. K., Ecological Studies, 208, Springer, Berlin, 207–230, doi:10.1007/b82392_13.

Scheu S. (1997) Effects of litter (beech and stinging nettle) and earthworms (Octolasion lacteum) on carbon and nutrient cycling in beech forests on a basalt-limestone gradient: A laboratory experiment. Biology and Fertility of Soils, 24, 384–393, doi:10.1007/s003740050262.

Schweizer M., Fear J., Cadish G. (1999) Isotopic (^{13}C) fractionation during plant residue decomposition and its implications for soil organic matter studies. Rapid Communication in Mass Spectrometry, 13, 1284–1290.

Steinmann K., Siegwolf R. T. W., Saurer M., Körner C. (2004) Carbon fluxes to the soil in a mature temperate forest assessed by ^{13}C isotope tracing. Oecologia, 141, 489–501, doi: 10.1007/s00442-004-1674-4.

Subke J. A., Hahn V., Battipaglia G., Linder S., Buchmann N., Cotrufo M. F. (2004) Feedback interactions between needle litter decomposition and rhizosphere activity. Oecologia 139, 551–559. doi:10.1007/s00442-004-1540-4.

Sulzman E. W., Brant J. B., Bowden R. D., Lajtha K. (2005) Contribution of aboveground litter, belowground litter, and rhizosphere respiration to total soil CO_2 efflux in an old growth coniferous forest. Biogeochemistry, 73, 231–256, doi: 10.1007/s10533-004-7314-6.

Swanston C. W., Torn M. S., Hanson P. J., Southon J. R., Garten C. T., Hanlon E. M., Ganio L. (2005) Initial characterization of processes of soil carbon stabilization using forest stand-level radiocarbon enrichment. Geoderma, 128, 52–62, doi:10.1016/j.geoderma.2004.12.015.

Thürig E., Palosuo T., Bucher J., Kaufmann, E. (2005) The impact of windthrow on carbon sequestration in Switzerland: a model-based assessment. Forest Ecology and Management, 210, 337–350, doi:10.1016/j.foreco.2005.02.030.

Tipping, E. (2002) Cation binding by humic substances, University Press, Cambridge, UK.

Tuzson B., Henne S., Brunner D., Steinbacher M., Mohn J., Buchmann B., Emmenegger L. (2010) Continuous isotopic composition measurements of tropospheric CO_2 at Jungfraujoch (3580m a.s.l.), Switzerland: real-time observation of regional pollution events. Atmospheric Chemistry Physics Discussion, 10, 24563–24593.

Tuzson B., Mohn J., Zeeman M. J., Werner R. A., Eugster W., Zahniser M. S., Nelson D. D., McManus J. B., Emmenegger L., (2008) High precision and continuous field measurements of $\delta^{13}C$ and $\delta^{18}O$ in carbon dioxide with a cryogen-free QCLAS. Applied Physics B. Lasers – Optics 92, 451–458, doi:10.1007/s00340-008-3085-4.

Vávřová P., Penttila T., Laiho, R. (2009) Decomposition of Scots pine fine woody debris in boreal conditions: implications for estimating carbon pools and fluxes. Forest Ecology and Management, 257, 401–412, doi:10.1016/j.foreco.2008.09.017.

Washington W. M., Knutti R., Meehl G. A., Teng H., Tebaldi C., Lawrence D., Buja L., Strand W. G. (2009) How much climate change can be avoided by mitigation?. Geophysical Research Letters, 36, L08703, doi:10.1029/2008GL037074.

Part B Publications

Paper I

Decomposition pathways of ^{13}C-depleted leaf litter in forest soils of the Swiss Jura

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Abstract

Decomposition of leaf litter and its incorporation into the mineral soil are key components of the C cycle in forest soils. In a ^{13}C-tracer experiment, we quantified the pathways of C from decomposing leaf litter in calcareous soils of a mixed beech forest in the Swiss Jura. Moreover, we assessed how important the cold season is for the decomposition of freshly fallen leaves. The annual C loss from the litter layer of 69–77% resulted mainly from the C mineralisation (29–34% of the initial litter C) and from the transfer of litter material to the deeper mineral soil (> 4 cm) by soil fauna (30%). Although only 4–5% of the initial litter C was leached as dissolved organic carbon (DOC), this pathway could be important for the C sequestration in soils in the long term: The DOC leached from the litter layer was mostly retained (95%) in the first 5 cm of the mineral soil by both physico-chemical sorption and biodegradation, and thus it might have contributed significantly to the litter-derived C recovered in the heavy fraction (> 1.6 g cm^{-3}) at 0–4 cm depth (4% of the initial litter C). About 80% of the annual DOC leaching from the litter layer occurred during the cold season (Nov–April) due to an initial DOC flush of water-soluble substances. In contrast, the litter mineralisation in winter accounted for only 25% of the annual C losses through CO_2 release from the labelled litter. Nevertheless, the highest contributions (45–60%) of litter decay to the heterotrophic soil respiration were observed on warm winter days when the mineral soil was still cold and the labile litter pool only partly mineralised. Our ^{13}C tracing also revealed that: (1) the fresh litter C only marginally primed the mineralisation of older SOM (> 1 yr); and (2) non-litter C, such as throughfall DOC, contributed significantly to the C fluxes from the litter layer since the microbial biomass and the DOC leached from the litter layer contained 20–30% and up to 60% of unlabelled C, respectively. In summary, our study shows that significant amounts of recent leaf litter C (< 1 yr) are incorporated into mineral soils and that the cold season is clearly less important for the litter turnover than the warm season in this beech forest ecosystem.

Keywords: Stable isotopes, Litter contribution, Soil CO_2 effluxes, Dissolved organic carbon, Priming effect, Winter, Beech forest

1. Introduction

The litter layer links the above- and belowground C cycle and is the C pool with the fastest turnover rates in forest soils. Although recent leaf litter (< 1 yr) generally accounts for less than 5% of the total amount of organic C in forest soils (Potter & Klooster, 1997), its mineralisation can contribute temporally up to 40% (Subke et al., 2004; Cisneros-Dozal et al., 2006) and annually more than 20% (Rey et al., 2002; Sulzman et al., 2005) to soil respiration. Moreover, the input of labile litter C may affect the soil respiration indirectly by priming the mineralisation of older, stable soil-organic matter (SOM) (Kuzyakov et al., 2000; Fontaine et al., 2007). A substantial fraction of litter-derived C is leached from decomposing litter (Hagedorn & Machwitz, 2007). In the mineral soil, this 'new' dissolved organic carbon (DOC) might be effectively stabilized by the interaction with mineral surfaces (Neff & Asner, 2001; Kalbitz & Kaiser, 2008). Finally, litter C is transformed into SOM and can persist for years or even decades, for instance occluded in aggregates (Swanston et al., 2005; Six et al., 2002). All of these processes in and directly below the litter layer may respond particularly sensitive to climatic changes due to the high lability of the litter C pool and the very high temperature and moisture variability in soils at the surface (Borken et al., 2003; Cisneros-Dozal, 2006; Joos et al., 2010). Therefore, the rates at which leaf litter is decomposed and transformed into different fractions of SOM are important parameters in soil carbon models (e.g. YASSO; Liski et al., 2005).

For a large number of ecosystems, litterbags have been used to estimate the control of the mass loss from litter by litter quality, decomposer communities and climatic conditions (e.g. Hättenschwiler et al., 1999; Moore et al., 1999; Liski et al., 2003). However, only a few field studies, tracking the fate of ^{13}C- or ^{14}C-labelled litter, have investigated the different pathways of litter decomposition; including mineralisation, leaching, and transformation into SOM (e.g. Bird & Torn, 2006; Fröberg et al., 2009; Rubino et al., 2010).

Using isotopes to track litter-derived C has several advantages over litterbags, such as: (1) litter-feeding soil fauna is not excluded from the decomposition process; (2) the downward transport of litter-derived C can be followed; and (3) the momentary litter-derived CO_2 effluxes can be measured, providing an insight into short-term dynamics of litter mineralisation. Recent tracer studies indicate that the fate of litter C may differ considerably in different forest ecosystems. For instance, while mineralisation was the most important decomposition pathway in a French beech forest (Ngao et al., 2005), the fraction of litter C transported to the mineral soil was twice as high as the fraction respired as CO_2 in an Italian poplar forest (Rubino et al., 2010).

Information about litter C dynamics is especially sparse for forests with calcareous bedrock as most studies on the cycling of litter-derived C have been conducted in acidic forest soils (e.g. Subke et al., 2004; Fröberg et al., 2007). One common characteristic of calcareous soils is that they have thin organic layers, which indicate a rapid loss of incoming litter due to a high level of biological activity (Scheu, 1997; Walthert et al., 2004). Results from microcosm studies suggest that, in base-rich soils, large amounts of fresh leaf litter are incorporated into the mineral soil by macrofauna within a few months (Scheu, 1997; Bonkowski et al., 1998). Without using an isotopic label, however, it is not possible to determine how quantitatively important this pathway is.

Although in deciduous forests, most leaf litter falls in autumn, little is known about the fate of this fresh litter C over the winter months. Is the litter preserved due to the cold temperatures or partly mineralised due to its high decomposability? Litterbag studies suggest that substantial amounts of freshly fallen litter C may already be lost in winter (e.g. Heim & Frey, 2004). The C losses observed in these studies, however, probably resulted largely from an initial DOC flush, which has been found to occur in several leaching experiments (Hagedorn & Machwitz, 2007; Hansson et al., 2009). The biodegradation of this 'wintertime' DOC in the mineral soil might be small as the soil microbial activity is low. Thus, the cold season could be an important period for the transport of litter-derived DOC to the mineral soil where it may be stabilized through interactions with mineral surfaces.

In this study, we present results from a litter manipulation experiment in which, at the beginning of the cold season, ^{13}C-labelled beech leaves were added to two adjacent forest soils with pH values of 7.5 and 5.9. The main goal of our ^{13}C-tracer study was to quantify the different pathways of litter-derived C in base-rich soils during one year: its release as CO_2, its leaching as DOC, its incorporation into the microbial biomass and its transport to the mineral soil. In particular, we aimed to assess: (1) the fate of freshly fallen litter C during the cold season; (2) the contribution of mineralisation and leaching of litter C to the C fluxes in forest soils throughout the year; (3) the retention of litter-derived C in the mineral soil; and (4) whether fresh litter C primes the decomposition of older soil C.

2. Materials and Methods

2.1. Study site

The litter experiment was established in a mixed beech forest at 680m a.s.l. on the steep south-facing slope (24°) of the Lägeren mountain close to Zurich (47°28’40.8’’ N, 8°21’55.2’’). At this Swiss CarboEurope research site (CH-Lae), the net-ecosystem CO_2

exchange has been measured routinely since 2004 using the eddy covariance method and soil respiration since 2006 using closed soil-chamber systems (Ruehr et al., 2009; Etzold et al., 2010). The site is on the geological transition between Jurassic limestone and Tertiary molasse sediments (Heim et al., 2009). The mean annual temperature is 8.4°C and the mean precipitation is 930 mm. The litter experiment was performed on two soil types 200 m apart. One of the soils was a Rendzic Leptosol (or Rendzina; pH = 7.5) and the other a Haplic Cambisol (pH = 5.9), according to the World Reference Base of Soil Resources (IUSS Working Group WRB, 2007). The bedrock of both soils was marl, but overlaid with limestone debris in the Rendzina. Both soils had mull-type organic layers indicative for a high biological activity. The properties of the topsoils (0–10 cm) are given in Table 1. Beech and Norway spruce dominated on both sites, but only the Rendzina was covered by a dense herb layer of wild garlic (*Allium ursinum* L.) in spring.

Table 1. *Properties of the top 0–10 cm of soil. Five soil cores (5 cm diameter) were taken from both soil types. The values are means ± standard errors.*

	pH ($CaCl_2$)	Particle-size distribution (%)			Fine-earth bulk density (g cm^{-3})	C_{org} (%)	C/N	C_{org} pool (kg m^{-2})	$\delta^{13}C_{org}$ (‰)
		250–2000 µm	2–250 µm	< 2 µm					
Rendzina	7.5 (0.1)	25 (2)	21 (3)	54 (5)	0.91 (0.03)	3.9 (0.3)	12.0 (0.1)	3.6 (0.2)	–27.2 (0.2)
Cambisol	5.9 (0.1)	23 (4)	35 (2)	42 (3)	0.94 (0.6)	2.8 (0.5)	11.3 (0.5)	2.6 (0.1)	–26.7 (0.2)

2.2 Labelled litter experiment

After leaf fall in mid November 2007, we replaced the native litter layer with ^{13}C-labelled beach leaves (750 g m^{-2}, $\delta^{13}C$ = –40.8‰, C/N = 28) in plots of 50 × 50 cm. The labelled litter originated from the final harvest of an open-top chamber experiment in Switzerland where beech trees were fumigated with ^{13}C-depleted CO_2 for four consecutive years (Hagedorn et al., 2005). Nearby each 'soil + litter' treatment (< 1 m), an identical surface area was left without any litter layer for the 'bare soil' treatment. Here, polystyrene shreds were added to mimic a litter layer and its impact on soil moisture and temperature. Both treatments were applied in five replicates to each of the two soil types, which were arranged within a radius of 10 m. The 'soil + litter' plots and the 'bare soil' plots were enclosed within acrylic glass frames (height 12 cm), which were inserted 2 cm into the forest floor and covered with a polyethylene net (mesh size = 0.7 × 0.3 mm) to prevent litter loss due to wind and inputs of fresh litter. In order to recognize the ^{13}C signal of litter-derived CO_2 better, we minimized

root respiration by digging a 30 cm deep trench around each plot. A plastic foliar was inserted to prevent external root ingrowths. Vegetation growth within the frames was suppressed by periodically weeding.

2.3 Soil CO_2 efflux and its $\delta^{13}C$

Soil CO_2 effluxes were measured at bi-weekly intervals with the chamber of a portable infrared gas analyzer (Li-8100, LI-COR Inc., Lincoln, NE, USA). This was placed on permanently installed PVC collars (5 cm high, 20 cm diameter), inserted to 2 cm depth. The measurements started one month before litter addition and were always carried out between 11 am and 4 pm.

On ten sampling days, the $\delta^{13}C$ of the soil respiration ($\delta^{13}C_{resp}$) was determined using the static closed soil-chamber approach (e.g. Ohlsson et al., 2005). The collars were closed with a plastic lid and one gas sample was collected from each chamber after a certain closure time, varying between 8 and 40 min. The closure time was estimated from the previous CO_2-efflux measurement to obtain an increase in the CO_2 concentration of about 400 ppm. The concentrations and the $\delta^{13}C$ of ambient CO_2 needed to calculate $\delta^{13}C_{resp}$ were determined from gas samples taken next to each collar immediately after they were closed. The gas samples were taken with a syringe through a septum in the lid and injected into glass vials (12 ml) previously evacuated and closed with an airtight rubber septum. Their ^{13}C ratios and the CO_2 concentrations were then analysed with a Gasbench II, connected to a mass spectrometer Delta Plus (both Thermo Finnigan Mat, Bremen, Germany).

The temperatures in the air, in the litter layer and at soil depths of 5 cm and 10 cm were measured using a Licor thermocouple for each sampling location at the same time as the CO_2 effluxes. To record soil temperatures continuously, temperature loggers (ibuttons, Maxim Integrated Products DS1922L, USA) were installed in three replicates per treatment at a soil depth of 10 cm.

2.4 DOC fluxes

Throughfall was sampled 1.5 m above the forest floor using PE funnels (Ø 11 cm) connected to 1.5-L PE bottles. The water percolating through the litter layer was captured with zero-tension lysimeters (13 × 17 cm PVC boxes), equipped with four openings (Ø 1cm) to allow soil animals to feed on the litter. Suction plates (Ø 5.5 cm) made of borosilicate glass (pore size P5; Schmizo, Zofingen, Switzerland) were used to collect the soil solution at depths of 5 cm and 10 cm (only 'soil + leaves'), applying a constant suction of 400 hPa with a vacuum

pump (EcoTech, Bonn, Germany). The soil water was collected in 0.5 L bottles buried in the soil. The water samples were collected after every larger rain event to minimize biodegradation of DOC. All water samples were passed through 0.45-µm cellulose-acetate filters (Schleicher & Schuell, ME25), pooled on a monthly base and refrigerated until analysis. This did not alter the DOC concentrations. HCl suprapur (30%) was added to all samples to remove inorganic C. Samples were then analysed for DOC concentrations, employing a TOC/TN analyzer (TOC-V, Shimadzu Corporation, Tokyo, Japan). In addition, the molar UV absorptivity at 285 nm in the DOC was measured using a Cary 50 UV-spectrophotometer (Varian, Palo Alto, USA). Aliquots (50–80 ml) were freeze-dried to determine the $\delta^{13}C$ of the DOC. Here, the addition of 5 mg of K_2SO_4 per sample facilitated the recovery and the weighing of the dissolved organic matter after freeze-drying.

2.5 Sampling and chemical analyses

Soil and litter samples: One year after the litter addition, the litter that remained on the soil surface was collected, cleaned to remove mineral particles and dried at 60°C for analysis. Subsequently, a soil core (Ø 5 cm) 10 cm in length was taken from each plot, frozen and divided into layers 2 cm thick with a hacksaw. The first two layers (0–2 cm, 2–4 cm) were physically fractionated into different SOM pools, while the soils from the other depths were freed from the roots, dried at 60°C and sieved (< 2mm) for total pool estimates.

Physical fractionation: Soils were fractionated into the light fraction (LF) and the heavy fraction (HF). At first, the dried soil samples were suspended in a sodium-polytungstate solution with a density of 1.6 g cm^{-3} (Kaiser & Guggenberger, 2007). After decanting the floating fraction (free LF), the suspension was ultrasonicated at 270 J ml^{-1} (HD3200, Bandelin, Zurich, Switzerland) to yield the occluded LF (Roscoe et al., 2000). To reduce the number of samples, the occluded LF and the free LF were combined. Samples of the LF and the HF were dried at 60°C, weighed and milled with a ball mill. Prior to the C analysis, all soil samples were additionally fumigated with acidic vapour for eight hours to remove inorganic C (Walthert et al., 2010).

Microbial biomass: We used the chloroform-fumigation extraction to determine the microbial biomass in the mineral soil at 0–2 cm depth and in the litter layer 4, 8 and 12 months after litter addition (Brooks et al., 1985). Soil samples were taken with a corer (Ø 2 cm) and within five hours after soil sampling, roots were removed and ten grams of fresh soil and five grams of litter was fumigated for 24 h with $CHCl_3$ and then extracted for one hour with 50 ml of 0.25 *M* K_2SO_4. Meanwhile, a second sample was extracted without fumigation. The organic C

content in the extracts was determined with a TOC analyzer (TOC-500, Shimadzu Corporation, Tokyo, Japan). The microbial C was then calculated from the difference between the fumigated and the unfumigated extracts, assuming an extraction efficiency (K_{ec}) of 0.45 (Wu et al., 1990). For the isotope analysis, the extracts were freeze-dried. The concentrations and the isotope ratios of C and N in the soil and freeze-dried samples were measured with an elemental analyzer (Euro EA 3000, HEKAtech, Germany) coupled to an isotope ratio mass spectrometer (Delta V Advantage, Thermo, Germany).

2.6 Calculations and statistics

$\delta^{13}C$ of soil-respired CO_2: Gas samples from each soil chamber represented a mixture of ambient CO_2 and cumulated soil-respired CO_2. The $\delta^{13}C$ of soil respired CO_2 ($\delta^{13}C_{resp}$) was calculated as follows (see Subke et al., 2004):

$$\delta^{13}C_{resp} = (\delta^{13}C_{chamber} \times CO_{2\ chamber} - \delta^{13}C_{ambient} \times CO_{2\ ambient}) / (CO_{2\ chamber} - CO_{2\ ambient}) \quad (1)$$

Litter-derived C: The contribution of labelled litter C (f_{litter}) to soil-C fluxes and pools was calculated for each plot individually using the following mixing model:

$$f_{litter} = (\delta^{13}C_{soil+litter} - \delta^{13}C_{control}) / \Delta^{13}C; \quad (2)$$

where $\delta^{13}C_{soil+litter}$ is the $\delta^{13}C$ of the C fluxes and pools in the 'soil + litter' treatment, $\delta^{13}C_{control}$ is the corresponding ^{13}C signature measured in the adjacent 'bare soil' plot and $\Delta^{13}C$ is the difference in the $\delta^{13}C$ between the bulk litter (–40.8‰.) and the soil organic C (SOC; –26.7 to –27.8‰). This approach assumes that isotopic fractionation of ^{13}C was minimal, or at least the same, in the litter layer and the mineral soil during both C mineralisation and DOC production (e.g. Schweizer et al., 1999; Santruckova et al., 2000; Fröberg et al., 2007).

DOC fluxes: The vertical fluxes of DOC below the litter layer and at depths of 5 and 10 cm were estimated by multiplying the DOC concentrations with water fluxes which had been simulated by employing the COUP model (Jansson & Karlberg, 2001). The organic C content and the particle-size distribution of different soil layers were used among other variables to parameterise the model. The climatic input variables – air temperature, precipitation, vapour pressure, wind speed and net radiation – were all recorded at a nearby meteorological station 100 m away.

Modelling CO_2 effluxes: The relation between soil CO_2 effluxes and soil temperature was fitted with the temperature function proposed by Fang & Moncrieff (2001):

$$CO_{2\ soil} = a \times (T - T_{min})^b; \quad (3)$$

where *T* is the soil temperature at a depth of 10 cm, and T_{min}, *a*, and *b* are parameters derived from non-parametric curve fits (Origin 7.1, OriginLab, USA). The annual C losses through CO_2 release from soils were estimated using the daily soil temperatures as input variables in Eq. 3 fitted to each plot separately.

It was not possible to fit the litter-derived CO_2 effluxes to a reasonable temperature function because the litter C pool declines with time. Alternatively, most ^{13}C-tracer studies simply interpolate the flux rates between the measurements without taking the temperature into consideration (e.g. Ngao et al., 2005; Bird & Torn, 2006). In this study, however, we employed a new approach to model litter-derived CO_2 effluxes more accurately by using the temperature dependency of litter-free soils and by incorporating the declining decomposability of the litter C. The temperature dependency of the mineral-soil respired CO_2 was estimated by fitting Eq. 3 to the flux rates in the 'bare soil' treatment. Assuming that the mineralisation of 'new' litter C and mineral-soil C are equally temperature sensitive, we scaled Eq. 3 to the litter-derived CO_2 effluxes at the beginning of January by linear transformation:

$$CO_{2\ litter} = a \times (T - T_{min})^b \times S \quad (4)$$

The transformation factor *S* was the theoretical ratio of litter-derived CO_2 and mineral soil-derived CO_2 at identical soil and air temperatures. The litter-derived CO_2 effluxes in January were selected as reference values because they contributed most to the soil respiration. Using the air temperature in Eq. 4, we calculated theoretical flux values for all sampling days. The ratio (factor *P*) between the measured litter-derived fluxes and the theoretical values describes then the change in the mineralisation potential of the litter C pool relative to the reference measurements. Therefore, *P* can be used as a correction factor in Eq. 4:

$$CO_{2\ litter} = a \times (T - T_{min})^b \times S \times P \quad (5)$$

After linearly interpolating *P* between the sampling days and using the daily air temperatures in Eq. 4, we were able to estimate the daily C losses from the litter through CO_2 release for every plot individually.

Statistics: Differences in C fluxes between the two litter treatments and the two soil types were tested with linear mixed effect models using the nlme package from the statistic software R version 2.8.1 (Pinheiro et al., 2008). By including random effects for the 'plot group' and for each single 'litter plot', the models accounted for both the split unit design of

the experiment and the repeated measurement structure. In all final models, normality and homoscedasticity of the residuals were verified visually with diagnostic plots and, when necessary, the dependent variable was log transformed.

3. Results

3.1 CO_2 effluxes

The soil respiration showed a pronounced seasonal pattern (Fig. 1), largely following the soil temperature at a depth of 10 cm (R^2 = 0.85–0.97; Eq. 3). No relationship, however, was found between soil CO_2 effluxes and soil water contents. This indicates that soil moisture ranging from 25 to 40 vol-% at a depth of 10 cm was not a limiting factor for microbial activity in mineral soils throughout the experiment. While no significant site effect (p = 0.25) on soil

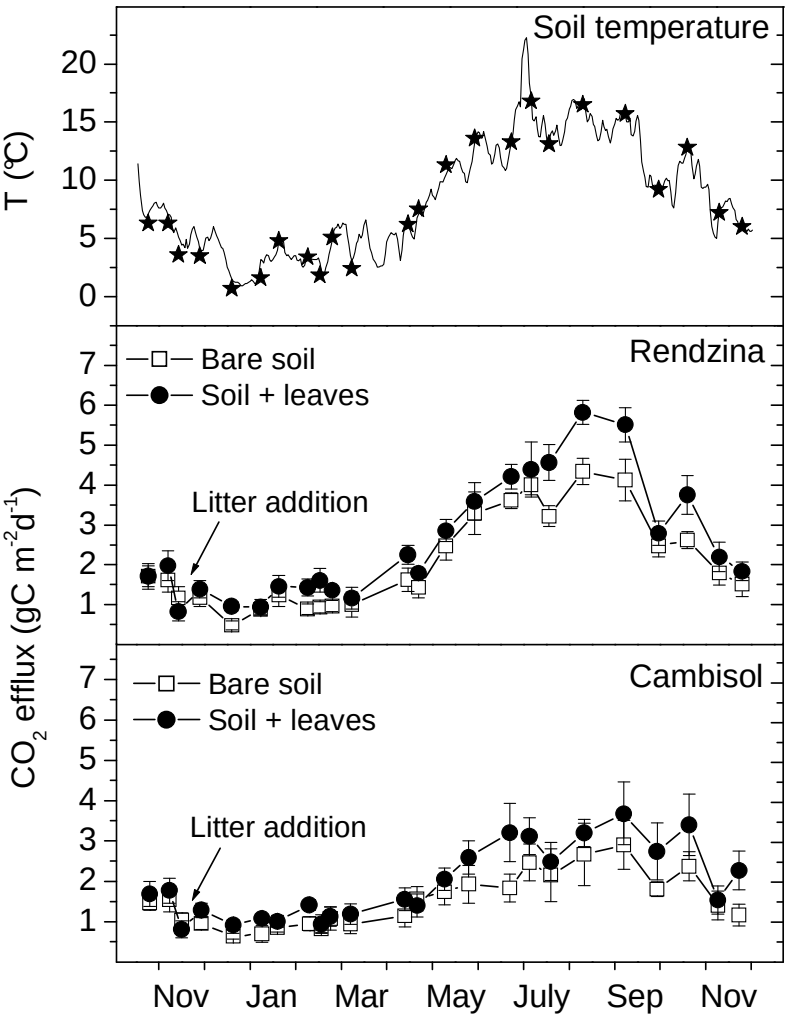

Figure 1. *Seasonal course of the soil temperature at a depth of 10 cm and of the soil CO_2 effluxes in both soils. The stars are the mean soil temperatures during the CO_2-efflux measurements. The CO_2 effluxes are the means of five replicates (± standard error).*

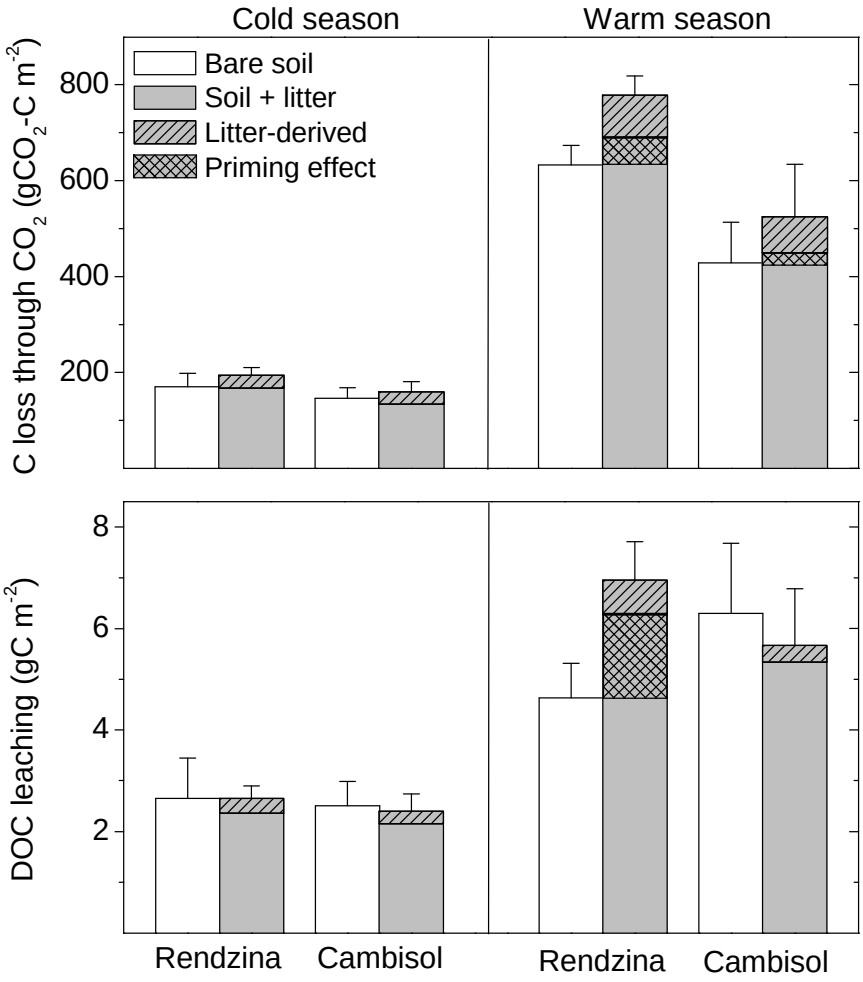

Figure 2. *C loss through CO_2 release and leaching of DOC at a depth of 5 cm in the 'bare soil' and the 'soil + litter' treatments, cumulated over the warm and the cold season. The crossed area indicates positive priming effects of the added litter on the mineralisation and the leaching of 'old' C (> 1 yr) in the mineral soil. All values are means of five replicates (± standard error).*

respiration was observed in winter (November 07–April 08), the soil CO_2 effluxes were, on average, 50% higher in the Rendzina than in the Cambisol ($p < 0.001$) during the warm season (April 08–November 08). Cumulated over one year, the mineral soils from the trenched plots lost 600–900 g C m^{-2} through microbial respiration (Fig. 2).

The natural ^{13}C abundance in mineral soil-derived CO_2 ranged from –24.0‰ to –27.5‰ in both the Rendzina and the Cambisol (Fig. 3a), indicating that the dissolution of carbonates contributed negligibly to the soil CO_2 effluxes. The addition of ^{13}C-depleted leaves ($\Delta^{13}C$= –13.6‰) enhanced CO_2 effluxes significantly (Fig. 1; $p < 0.001$), and decreased the ^{13}C ratio of soil-respired CO_2 by 1.2–8.4‰ relative to the 'bare soil' (Fig. 3a). The only exception was the sampling in December at air temperatures of –4°C when no litter-derived CO_2 effluxes were

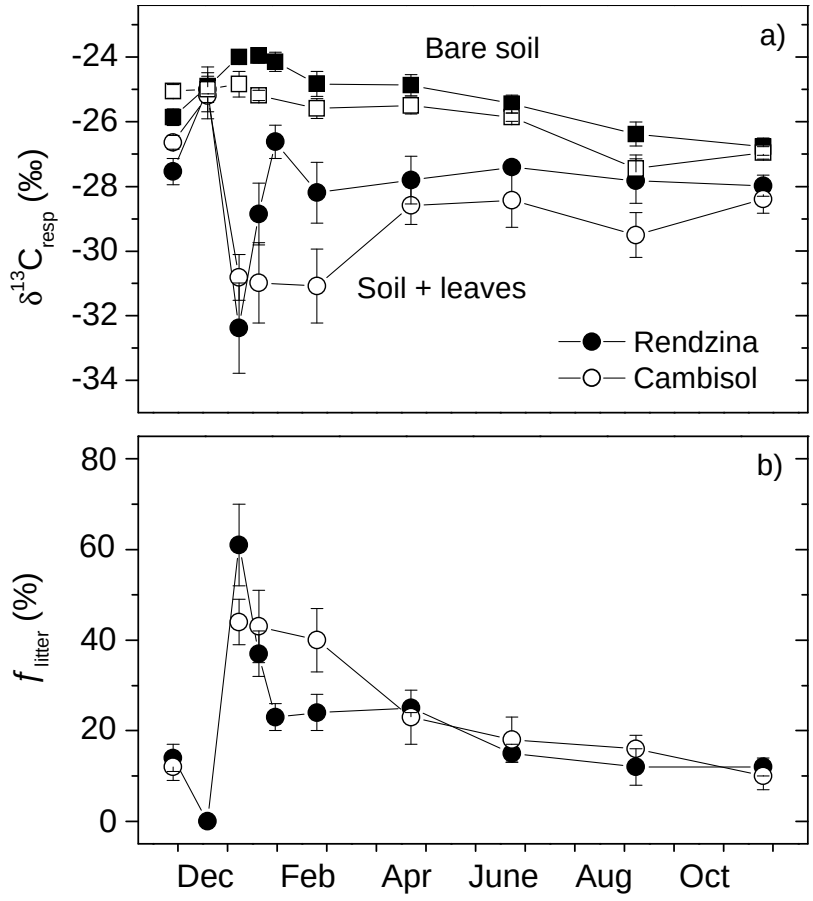

Figure 3. a) *^{13}C signatures of soil CO_2 effluxes ($\delta^{13}C_{resp}$), and* **b)** *contributions of litter-derived C to the heterotrophic soil respiration (f_{litter}). The values are the means of five replicates (± standard error).*

observed. Three weeks later, however, at air temperatures of 6°C and soil temperatures of about 1°C, the contribution of leaf litter to soil-respired CO_2 (f_{litter}) peaked at 60% in the Rendzina and 45% in the Cambisol (Fig. 3b). Subsequently, f_{litter} declined continuously to about 10% at the end of the experiment in November.

The seasonal pattern of the litter mineralisation was less pronounced than that of the soil respiration (Fig. 4): The highest litter-derived CO_2 effluxes in winter were only 25% lower than the peaking fluxes in summer, despite differences in air temperatures of 13°C. In comparison, the peaks in total soil-respiration rates differed by a factor of 2.5 between the seasons (Fig. 1). Soil type had a minor effect on the mineralisation rates of the litter C. They were slightly (–15%), but not significantly ($p = 0.30$), lower in the Cambisol than in the Rendzina. The annual C losses of the litter, estimated by applying the temperature dependency of the CO_2 effluxes in the 'bare soil' treatment ($R^2 = 0.91$, see Eq. 3 and 5), were 33.5 ±4.5% in the Rendzina and 29.0 ±3.3% in the Cambisol. Mineralisation during the five winter months accounted for 25% of the annually respired litter C (Table 2; Fig. 4).

Table 2. *Different pathways of litter-derived C. The C fluxes were either modelled (CO_2) or cumulated (DOC) over five winter months and over the entire year. The litter C that remained in the litter layer or was incorporated in either the light fraction (LF < 1.6 g cm^{-3}) or the heavy fraction (HF) of the mineral soil at 0–2 cm depth was determined one year after litter addition. The values are means and standard errors from five plots.*

		C fluxes (% of initial litter C)				C pools (% of initial litter C)		
	Period	CO_2	DOC Oi	DOC 5 cm	DOC 10 cm	LF 0–2 cm	HF 0–2 cm	Litter layer
Rendzina	Winter	7.9 (0.8)	2.9 (0.6)	0.08 (0.0)	0.06 (0.0)			
	1 year	33.5 (4.5)	3.8 (0.7)	0.26 (0.1)	0.15 (0.0)	3.3 (1.3)	7.2 (2.0)	22.6 (3.3)
Cambisol	Winter	7.5 (0.7)	3.7 (0.2)	0.08 (0.0)	0.07 (0.0)			
	1 year	29.0 (3.3)	4.6 (0.3)	0.17 (0.1)	0.12 (0.0)	3.7 (1.3)	2.0 (1.6)	31.0 (10)

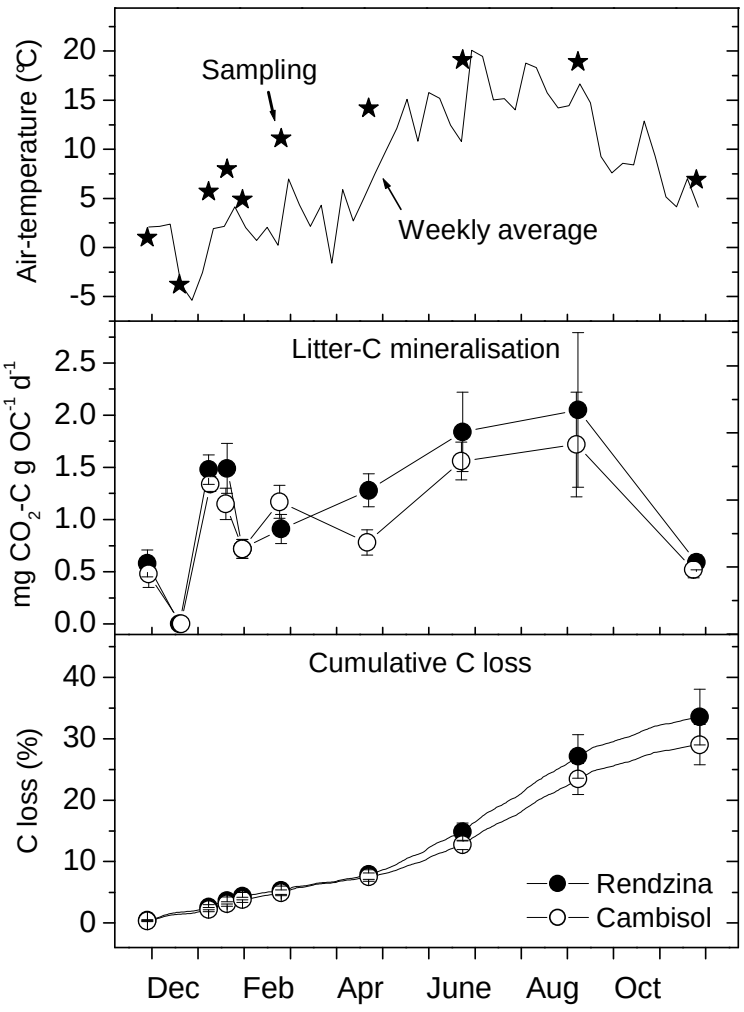

Figure 4. *Air temperature, rates of litter-C mineralisation and cumulative C loss through CO_2 release from the labelled leaves. The values are the means of five replicates (± standard error).*

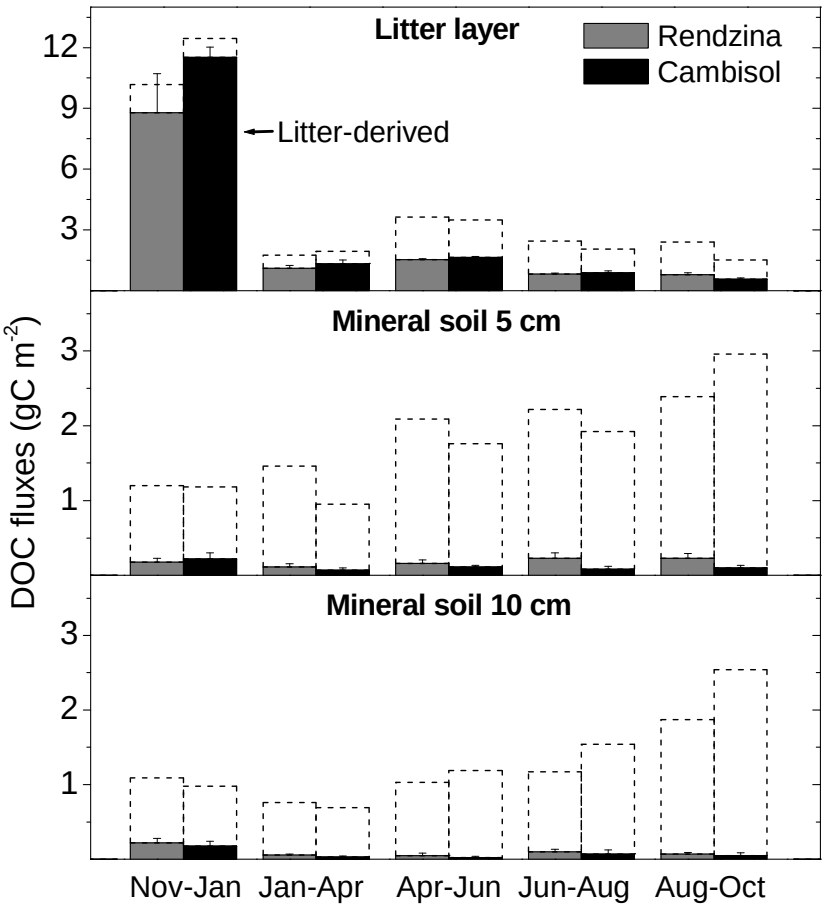

Figure 5. *DOC fluxes at three different depths. The entire bar represents the total DOC flux, which consists of litter-derived DOC (filled part) and non-litter DOC (dashed line). The values are the means of five replicates (± standard error).*

Fractions of soil-respired CO_2 that originated from priming effects were calculated as the difference between cumulated C losses through CO_2 release from the 'soil + litter' treatment and the sum of C losses from the litter layer and the 'bare soil' treatment (Fig. 2). These differences were small in winter, indicating that the litter layer had no effect on the CO_2 release from the mineral soil. During the warm season, however, the litter layer increased the SOM mineralisation slightly, but not significantly (+7%; $p = 0.21$).

3.2 DOC leaching and retention

Litter layer: The leaching of DOC from the litter layer significantly differed from the seasonal course of CO_2 effluxes. About 80% of the annual fluxes of litter-derived DOC occurred during the five winter months, mainly due to an initial DOC flush (Fig. 5). Subsequent to the

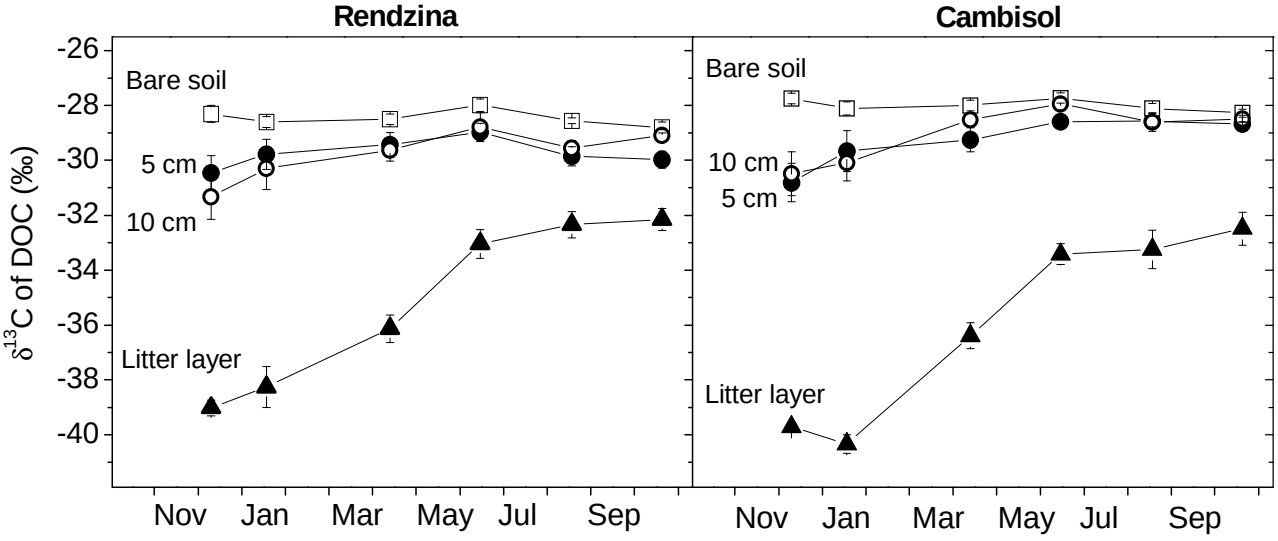

Figure 6. *^{13}C signature of the DOC leached from the litter layer and the mineral soil at depths of 5 cm (bare soil, soil + leaves) and 10 cm (soil + leaves). The values are the means of five replicates (± standard error).*

first leaching cycle, the fluxes of litter-derived DOC dropped to values about eight times lower and then remained in a narrow range throughout the experiment.

The ^{13}C ratio of the DOC leached from the labelled litter layer increased by 6–7‰ over the course of the experiment (Fig. 6). Thus, up to 60% of this DOC originated from non-litter C. For one year, the leaf litter lost 13–16 g C m^{-2} through DOC leaching. This amount corresponds to 4–5% of its initial C pool (Table 2) and to 11–16% of the litter C respired as CO_2. The DOC release from the litter did not depend on the soil type throughout the experiment (Fig. 5; $p = 0.27$). The DOC of the first leaching cycle was characterised by an approximately 40% lower molar UV absorptivity compared to the subsequently leached DOC, with absorptivity values ranging from 220 to 300 L cm^{-1} mol^{-1} (data not shown).

Mineral soil: The DOC fluxes, cumulated over one year and averaged for both soils, declined from 22 g DOC m^{-2} yr^{-1} under the litter layer, to 9 and 6.5 g DOC m^{-2} yr^{-1} at soil depths of 5 and 10 cm, respectively. The contribution of litter-derived DOC to mineral-soil DOC was largest in early winter, when it was 17–24% but it then dropped to a relatively constant value of about 10% in the Rendzina and about 5% in the Cambisol (Fig. 5 and 6). Thus, only small amounts of labelled litter DOC were recovered in the mineral soil (at 5 cm: 0.8 g DOC m^{-2} yr^{-1}; at 10 cm: 0.4 g DOC m^{-2} yr^{-1}; Fig. 5). This finding indicates that most DOC (93–98%)

Table 3. *Microbial biomass C and its proportion, derived from the 'new' litter C determined in the litter layer and the mineral soil (0–2 cm) using chloroform-fumigation extraction. The samples were collected 4, 8 and 12 months after litter addition. The values are means and standard errors from three plots.*

		Microbial C (mg g^{-1} SOC)			Litter-C fraction (%)		
Soil	Sample	March	July	Nov	March	July	Nov
Rendzina	Litter layer	18 (1)	33 (1)	28 (1)	80 (2)	92 (3)	82 (5)
	0–2 cm (below litter)	36 (3)	24 (2)	27 (1)	5 (1)	9 (4)	3 (6)
	0–2 cm (bare soil)	39 (3)	26 (2)	26 (4)	-	-	-
Cambisol	Litter layer	21 (3)	36 (3)	24 (3)	88 (3)	90 (4)	80 (5)
	0–2 cm (below litter)	64 (2)	21 (0)	30 (5)	3 (4)	5 (3)	7 (4)
	0–2 cm (bare soil)	41 (12)	18 (1)	27 (6)	-	-	-

leached from the litter layer was retained in the top centimetres of the soil profile.

While there was no soil-type effect on DOC fluxes from the mineral soil itself ($p = 0.69$), twice times as much litter-derived DOC was recovered at depths of 5 and 10 cm in the Rendzina than in the Cambisol (Fig. 5). This suggests a stronger retention of 'new' litter DOC in the slightly acidic mineral soil. The fact that the DOC fluxes at 5 cm in the 'bare soil' did not differ significantly from those in the 'soil + litter' treatment from November to April ($p = 0.71$) shows that, in winter, the litter layer barely stimulated the DOC production in the mineral soil (Fig. 2). During the warm season, the litter effect on the leaching of native DOC in the mineral soil depended on the soil type ($P_{litter \times soil} < 0.01$; Fig. 2): The fluxes of 'old' DOC in the 'soil+litter' plots were clearly higher (+35%) than in the 'bare soil' plots in the Rendzina, but slightly lower in the Cambisol (–15%).

3.3 Microbial C

The amount of microbial C (mg g^{-1} SOC) at a depth of 0–2 cm did not differ significantly between either the soil types ($p = 0.43$) or the 'bare soil' and the 'soil + litter' treatment ($p = 0.55$; Table 3). While in the litter layer the proportion of microbial C almost doubled from winter to summer (Table 3), the microbial C in the mineral soil decreased by about 30% from the cold to the warm season.

The ^{13}C ratios of the microbial biomass were about 4‰ higher than those of the light fraction (LF; <1.6 g cm^{-3}) and about 2.5‰ higher than those of the heavy fraction (HF; > 1.6 g cm^{-3}) at a depth of 0–2 cm (Fig. 7). In the litter layer, the microbial ^{13}C shift relative to the

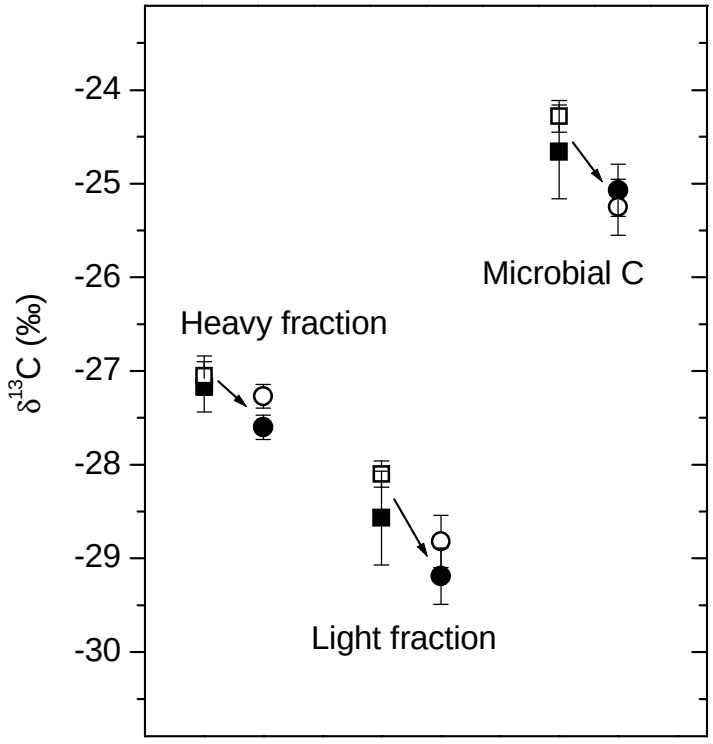

Figure 7. *Shift in the ^{13}C signature of different SOM fractions (0–2 cm) one year after the litter addition. The squares are the 'bare soil', the circles are the 'soil + litter' treatment, the filled symbols are the Rendzina and the open symbols are the Cambisol. The values are the means of five replicates (± standard error).*

bulk litter ranged from 5 to 6.5‰ throughout the experiment. Under the assumption that the native ^{13}C enrichment of microbial C on litter was at most 4‰ (see above microbial C vs. LF), we estimated that roughly more than 10–20% of the C assimilated by microbes in the litter layer did not originate from the labelled litter (Table 3). In the microbial biomass of the mineral soil at 0–2 cm depth, the fraction of litter-derived C ranged from 3 to 9% on all three sampling dates (Table 3). Hence, 1–2 g m^{-2} of litter C was incorporated into the microbial biomass at 0–2 cm depth, corresponding to about 0.5% of the total litter C added.

3.4 New C in different SOC pools

At the end of the experiment, the $\delta^{13}C$ of the litter collected from the soil surface was slightly, but not significantly, higher than the $\delta^{13}C$ of the initially added litter in both soils (–40.4‰ vs. –40.8‰; $p = 0.22$). The fraction of added leaf C that remained in the litter layer after one year was on average 23% in the Rendzina and 31% in the Cambisol (Table 2).

The $\delta^{13}C$ values in both the LF and the HF of the mineral soil at 0–2 cm depth were shifted slightly, but significantly, by the addition of litter ($p<0.001$; Fig. 7). One year after litter

addition, about 3.5% of the initial litter C was stored in the LF at 0–2 cm depth (Table 2), where it contributed 6% to the total C pool of the LF. The HF at 0–2 cm depth contained two times more 'old' C than the LF, and stored 7% of the initial litter C pool in the Rendzina and 2% in the Cambisol. No significant change in the ^{13}C signature, however, was observed at 2–4 cm depth in either the HF or the LF (data not shown).

4. Discussion

Tracing ^{13}C in litter-derived C provided a more detailed insight into the pathways of decomposing beech leaves than the analyses of net C fluxes in the litter layer and in the mineral soil. For instance, total DOC fluxes changed only slightly from the litter layer to the soil depth of 5 cm from spring to autumn (Fig. 5 and 8). This would suggest that processing of litter-derived DOC in the mineral soil was negligible. In contrast, the tracking of ^{13}C-labelled litter revealed that, during the warm season, 80–90% of the DOC leached from the litter was retained in the mineral soil, and 90–95% of the DOC at the depth of 5 cm originated from the mineral soil itself. Thus, the DOC turnover was much greater than expected from the net fluxes.

We also found that ‘external’ non-litter C contributed significantly to the C fluxes from the litter layer since the microbial biomass contained 10–20% of unlabelled C and the DOC leached from the litter layer up to 60% (Table 3; Fig. 5 and 6). Similar fractions of non-litter C were recently observed in C fluxes from ^{14}C-labelled litter in a hardwood forest (Fröberg et al., 2009). Our study suggests that one source of this non-litter C was throughfall DOC, which amounted to 5 g C m^{-2} yr^{-1}, and thus corresponded to the non-litter C observed in the DOC leached from the litter layer. The fraction of unlabelled C in the microbial biomass, however, indicates an input of non-litter C to the litter layer of more than 15 g m^{-2} yr^{-1}, which probably originated from the deposition of pollen and other particulate organic matter. Throughfall measurements in German beech forests by LeMellec et al. (2010) have shown that particulate organic matter can exceed DOC inputs.

4.1 Pathways of litter decomposition

After one year of decomposition, 29–34% of the litter-derived C had returned as CO_2 to the atmosphere and 4–5% had been leached as DOC (Table 2, Fig. 4). The sum of both fluxes was within the range of the annual C losses (24–44%) from beech leaves observed in litter bag studies in Switzerland (Hättenschwiler et al., 1999; Heim & Frey, 2004).

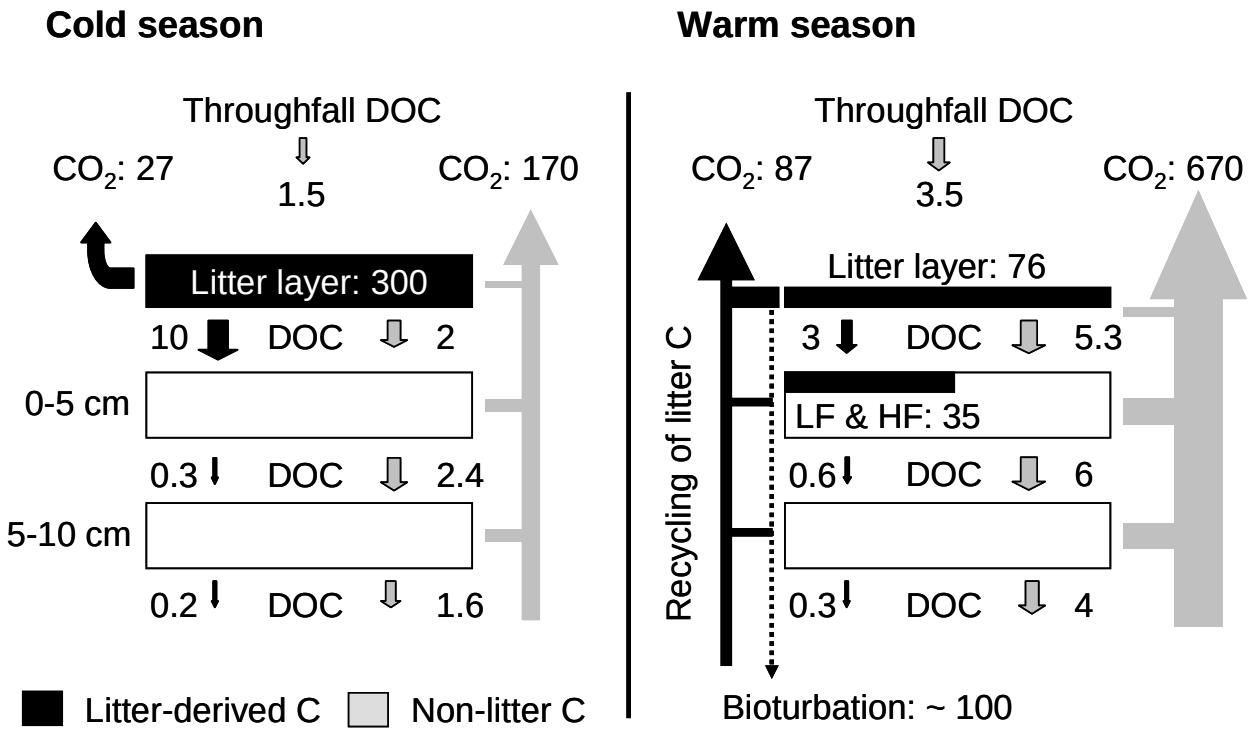

Figure 8. *C fluxes and C pools (g C m^{-2}) from added leaf-litter C and non-litter C, cumulated over the cold (Nov 07–April 08) and the warm season (April 08–Nov 08). The values are the means of five replicates in the Rendzina.*

In both soils, we recovered 70% of the labelled leaf litter C by summing up across all fluxes and pools that had been measured throughout the experiment (Table 2; Fig. 8). We attribute the missing litter C in the mass balance mainly to the transfer of leaf material by soil animals into deeper soil horizons. This was observed in a lab experiment on calcareous soils by Scheu (1997), who found that earthworms removed more than 30% of beech leaves within three months. Consequently, the export of leaf litter by soil fauna probably equalled the loss via mineralisation and exceeded the leaching of 'new' DOC from the litter layer, as well as the incorporation of 'new' C into the mineral soil at a depth 0–2 cm (Table 2; Fig. 8). Therefore, in beech forests with mull-type organic layers, bioturbation is the dominant transport pathway of 'new' litter C into the mineral soil, while leaching seems to be less important than in coniferous ecosystems with thick organic layers (e.g. Neff & Asner, 2001; Hagedorn et al., 2008; Kalbitz & Kaiser, 2008).

Our finding that the pathways of litter C differed only slightly between the Rendzina and the Cambisol (Table 2) suggests that the pH values of 7.5 and 5.9 are both within the optimum range for microbial decay of leaf litter and activity of the soil fauna. Thus, our study provides no support for the general assumption that litter decomposition is positively linked to the pH value associated with a higher species diversity of the decomposer community (Vesterdal, 1999; Schaefer et al., 2009).

4.2 Seasonal dynamics in mineralisation and leaching

The mineralisation and the leaching of litter C differed greatly not only quantitatively, but also in their seasonal dynamics. While respiration during the five winter months accounted for only 25% of the annual C loss through CO_2 from the litter (Table 2; Fig. 4 and 8), the DOC leaching in the cold season was 80% of the annual leaching losses (Table 2; Fig. 5). This result suggests that mineralisation and leaching from litter are not basically linked, which goes along with the findings of several lab studies that CO_2 and DOC production correlate only slightly (Magill & Aber, 2000; Park et al., 2002; Hagedorn & Machwitz, 2007). The large DOC fluxes in early winter probably resulted from the flushing out of water-soluble substances by heavy rainfall. The initially leached DOC had a low molar UV absorptivity, indicating that it comprised largely substances with a low-molecular weight and not microbially degraded aromatic compounds (Dilling & Kaiser, 2002). Although such peaking DOC concentrations have already been observed under litter layers following the autumn leaf fall (Park & Matzner, 2003), we cannot rule out that in our litter experiment, the DOC flush was intensified by the drying of the litter before its application (see Fröberg et al., 2007).

In several tracer studies, measuring litter-derived CO_2 effluxes between spring and autumn, the litter fractions of soil respiration (f_{litter}) have been observed to decline quickly with increasing time after litter addition (Rochette et al., 1999; Subke et al., 2004; Joos et al., 2010). Our one-year experiment starting in winter only partly confirms this pronounced temporal pattern (Fig. 3). The highest values for f_{litter} (up to 60%) were indeed measured in winter when the most labile components of the leaf litter were still available, while during the warm season, more than five months after the litter addition, f_{litter} was always below 30%. Our results, however, also revealed that in winter, f_{litter} considerably depends on the gradient between air and soil temperatures. The highest values for f_{litter} were observed on warm winter days when air temperatures exceeded 5°C but the temperatures in the mineral soil were still close to zero degrees. In comparison, in November and December, a very cold (0–1°C) or frozen litter layer on soils with temperatures above 3°C only contributed negligibly to soil

respiration despite the very fresh litter C (Fig. 3). Modelling the seasonal C losses through CO_2 from both the added litter and the mineral soil, taking these very cold periods into account, resulted in clearly higher C losses from the litter in the warm season than in the cold season (Fig. 2 and 8) and only slightly lower values for f_{litter} (13% vs. 15%). Thus, the warm season was clearly more important for the litter turnover than the cold season.

It should be noted that we may have slightly overestimated the cumulated C losses in summer because we measured the litter-derived CO_2 effluxes only on a few sampling days. Thereby, we ignored the few periods (at most 20 days) when the litter layer was completely desiccated. Nevertheless, our results are in line with those from a litterbag study in Switzerland in which beech leaves were found to have lost 24–40% of their initial weight after one year, but only 1–9% during the initial six winter months (Heim & Frey, 2004).

We assume that the litter C respired over the winter months originated largely from labile leaf compounds such as hydrophilic substances because: (1) the cumulated C losses through CO_2 release in winter of about 8% agreed well with the fraction of water-soluble components in beech leaves (Vesterdal, 1999; Zeller et al., 2000); and (2) the mineralisation rates declined by 30% from January to April despite a temperature increase of 9°C (Fig. 4), indicating the loss of the most labile compounds. Over the warm season, however, the decrease in the litter C pool was only slightly reflected in the litter-derived CO_2 effluxes. In particular, in late summer and in autumn, the recycling of litter C (< 1 yr) already incorporated into the mineral soil by DOC leaching, microbes or invertebrates might have been a significant CO_2 source (Fig. 8). At the last sampling in November, for instance, we observed that f_{litter} was about 15% in two litter plots where the litter layer had completely disappeared.

4.3 Retention and stabilisation of litter DOC in the mineral soil

The ^{13}C values showed a strong decline in litter-derived DOC from the litter layer to the mineral soil at depths of 5 and 10 cm (Fig. 5 and 6), indicating an effective retention of this 'new' DOC within the first centimetres of the mineral soil. The 'new' C accounted, on average, for only 10% of the DOC flux at 5 cm, which implies that most of the DOC leached below 5 cm originated from the mineral soil itself. Comparable strong retentions of ^{13}C- and ^{14}C-labelled litter DOC have been observed for both mineral soils (Fröberg et al., 2009) and organic layers (Fröberg et al., 2007; Müller et al., 2009), but the mechanisms behind them remain uncertain.

Our results provide evidence that both physico-chemical sorption and biodegradation contributed significantly to the DOC retention. We found that DOC was retained not only in

the warm season but also in winter, and thus also when microbial activity was low, which suggests that sorption processes played a crucial role. The enhanced DOC retention in the Cambisol (Fig. 5), which was possibly due to a stronger sorption to soil minerals at lower pH values (Tipping, 2002), supports this conclusion. On the other hand, the fact that the initially flushed DOC, which contained the largest hydrophilic fraction, was more strongly retained (98%) than the DOC subsequently leached (70–95%) suggests that DOC was also taken up by microbes since hydrophilic DOC has a lower affinity to mineral surfaces than hydrophobic DOC and is also more biodegradable (Kaiser & Guggenberger, 2000; Kalbitz et al., 2003). Indeed, on all three sampling dates, we found small but detectable fractions of 'new' litter C in the microbial biomass of the mineral soil at a depth of 0–2 cm (1–2 g C m^{-2}; Table 3 and Fig. 7). At the end of the winter, this new microbial C probably originated from litter-derived DOC since it appears very likely that the cold temperatures prevented the transport of litter material by invertebrates. Assuming that 50% of the litter-derived DOC assimilated by the microbial biomass was lost as CO_2 (Six et al., 2006), a rough mass balance indicates that 20–40% of the litter DOC could have been biologically immobilized in the two soil types at a depth of 0–2 cm during the winter months.

The retention of litter-derived DOC in the mineral soil either by microbial immobilisation or by physico-chemical interactions represents an important stabilization mechanism for SOM (Kaiser & Guggenberger, 2000; Kalbitz & Kaiser, 2008). At the end of our experiment, the heavy soil fraction at 0–2 cm depth did indeed comprise 4% new C in the Rendzina and 2% new C in the Cambisol. Although these fractions seem small, they corresponded to 25 g m^{-2} of new litter C in the Rendzina and 7 g m^{-2} in the Cambisol, which is in the range of the total DOC amount retained in the mineral soil (12–15 g DOC $m^{-2}yr^{-1}$). In the long-term, this pathway could contribute significantly to C sequestration in soils.

4.4 No priming of native C mineralisation

Labile litter C may stimulate the mineralisation of older stable SOM (Fontaine et al., 2007; Nottingham et al., 2009), but in our study we found only slight support for such a priming effect (Fig. 2). No priming occurred in winter, while during the warm season the leaf litter enhanced the SOM mineralisation slightly but not significantly (+7%). These small priming effects fit in the findings of Subke et al. (2004) that the litter layer had no effect on the mineralisation of 'old' SOM in forest soils where the root respiration was excluded by girdling. In contrast, Sulzman et al. (2005) reported that, after six years of additional leaf litter input, the mineralisation of older C, calculated from the difference in soil CO_2 effluxes

between double litter plots and control plots, was significantly stimulated (+20–30%). In our experiment, the incorporation of leaf litter into the mineral soil by soil fauna probably did not start before summer, and most litter-derived DOC was retained in the uppermost soil (Fig. 8). Hence, the contact of 'new' labile C with older SOM was largely restricted to the first centimetres of the soil during most of the experiment. This part of the soil probably made only a minor contribution to the totally respired CO_2, which, in turn, might explain the insignificant response of mineral soil-derived CO_2 to the fresh C source. Moreover, the addition of litter did not alter the microbial biomass of the mineral soil (Table 3), which could have affected the mineralisation of SOM (e.g. Nottingham et al., 2009). The marginal effect of litter on microbes is underlined by the small fractions of recent litter C recovered in the microbial C at a depth of 0–2 cm (3–9%, Table 3). This is further supported by the results from a ^{14}C-tracer study on the Oak Ridge Reservation, where 1–4 year old litter was only a small C source (< 10%) for microbes in the mineral soil (Kramer et al., 2010).

Recent tracer-based studies suggest that the supply of fresh DOC, such as throughfall DOC or rhizodeposits, can enhance the mobilization of native DOC in the first centimetres of the soil (Hagedorn et al., 2008; Müller et al., 2009). Our results, however, give a controversial picture: No priming effect was observed in winter (Fig. 2), while during the warm season, the leaching of native DOC in the mineral soil was increased under the litter layer in the Rendzina (+35%), but slightly reduced relative to the 'bare soil' in the litter plots of the Cambisol (–15%). Here, we cannot clarify whether the priming effect on DOC leaching indeed depends on the soil type possibly due to a different availability of nutrients (Fontaine et al., 2003), or if the different responses can simply be attributed to the spatial heterogeneity of the DOC leaching.

5. Conclusions

Using ^{13}C-labelled litter yielded insights into the fate of decomposing leaf litter in a mixed beech forest in the Swiss Jura. We quantified three main pathways of litter-derived C, which all corresponded to about 30% of the initial litter C pool: Litter-C mineralisation, transfer of litter material to the deeper mineral soil (> 4 cm depth) by soil fauna, and litter C remaining on the soil surface. Only 4–5% of the added litter C, however, was leached as DOC. Our study also shows that in these types of forest soils with high pH values: (1) the greatest contribution of fresh leaf litter to the soil respiration can be expected on warm winter days when the mineral soil is still cold and the labile litter pool is only partly mineralised; (2) about 25% of the annual litter mineralisation and 80% of the litter-derived DOC leaching occurred

during winter (Nov–April); (3) about 95% of the DOC leached from the litter layer was retained in the first centimetres of the mineral soil, probably due to both physico-chemical sorption and biodegradation; (4) 'external' non-litter C contributed significantly to the C fluxes from the litter layer; and (5) fresh fallen litter did not prime the mineralisation of old SOM.

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References

Bird J. A., Torn M. S. (2006) Fine roots vs. needles: a comparison of ^{13}C and ^{15}N dynamics in a ponderosa pine forest soil. Biogeochemistry 79, 361–382, doi:10.1007/s10533-005-5632-y.

Bonkowski M., Scheu S., Schaefer M. (1998) Interactions of earthworms (Octolasion lacteum), millipedes (Glomeris marginata) and plants (Hordelymus europaeus) in a beechwood on a basalt hill: implications for litter decomposition and soil formation. Applied Soil Ecology 9, 161–166, doi:10.1016/S0929-1393(98)00070-5.

Borken W., Davidson E. A., Savage K., Gaudinski J., Trumbore S. E. (2003) Drying and wetting effects on carbon dioxide release from organic horizons. Soil Science Society of America Journal, 67, 1888–1896, doi: 10.2136/sssaj2003.1888.

Brookes P. C., Landman A., Pruden G., Jenkinson D. S. (1985) Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass in soil. Soil Biology and Biochemistry, 17, 837–842.

Cisneros-Dozal L. M., Trumbore S., Hanson P. J. (2006) Partitioning sources of soil-respired CO_2 and their seasonal variation using a unique radiocarbon tracer. Global Change Biology, 12, 194–204, doi: 10.1111/j.1365-2486.2005.01061.x.

Dilling J., Kaiser K. (2003) Estimation of the hydrophobic fraction of dissolved organic matter in water samples using UV photometry. Water Research, 36, 5037–5044, doi:10.1016/S0043-1354(02)00365-2.

Etzold S., Buchmann N., Eugster W. (2010) Contribution of advection to the carbon budget measured by eddy covariance at a steep mountain slope forest in Switzerland. Biogeosciences 7, 2461–2475, doi:10.5194/bg-7-2461-2010.

Fang C., Moncrieff J. B. (2001) The dependence of soil CO_2 efflux on temperature. Soil Biology and Biochemistry, 33, 155–165, doi:10.1016/S0038-0717(00)00125-5.

Fontaine S., Mariotti A., Abbadie L. (2003) The priming effect of organic matter: a question of microbial competition?. Soil Biology and Biochemistry, 35, 837–843, doi:10.1016/S0038-0717(03)00123-8.

Fontaine S., Barot S., Barré P., Bdioui N., Mary B., Rumpel C. (2007) Stability of organic carbon in deep soil layers controlled by fresh carbon supply. Natur, 450, 277–280, doi:10.1038/nature06275.

Fröberg M., Berggren Kleja D., Hagedorn F. (2007) The contribution of fresh litter to dissolved organic carbon leached from a coniferous forest floor. European Journal of Soil Science, 58, 108–114, doi:10.1111/j.1365-2389.2006.00812.x.

Fröberg M., Hanson P. J., Trumbore S. E., Swanston C. W., Todd D. E. (2009) Flux of carbon from ^{14}C-enriched leaf litter throughout a forest soil mesocosm. Geoderma, 149, 181–188, doi:10.1016/j.geoderma.2008.11.029.

Hagedorn F., Maurer S., Bucher J. B., Siegwolf R. T. W. (2005) Immobilization, stabilization and remobilization of nitrogen in forest soils at elevated CO_2: a ^{15}N and ^{13}C tracer study. Global Change Biology, 11, 1816–182, doi:10.1111/j.1365-2486.2005.01041.x.

Hagedorn F., Machwitz M. (2007) Controls on dissolved organic matter leaching from forest litter grown under elevated atmospheric CO_2. Soil Biology and Biochemistry, 39, 1759–1769, doi:10.1016/j.soilbio.2007.01.038.

Hagedorn F., van Hees P. A. W., Handa I. T., Hättenschwiler S. (2008) Elevated atmospheric CO_2 fuels leaching of old dissolved organic matter at the alpine treeline. Global Biogeochemical Cycles, 22, GB2004, doi:10.1029/2007GB003026.

Hansson K., Berggren Kleja D., Kalbitz K., Larsson H. (2010) Amounts of carbon mineralised and leached as DOC during decomposition of Norway spruce needles and fine roots. Soil Biology and Biochemistry, 42, 178–185, doi:10.1016/j.soilbio.2009.10.013.

Hättenschwiler S., Bühler S., Körner C. (1999) Quality, decomposition and isopod consumption of tree litter produced under elevated CO_2. Oikos, 85, 271–281.

Heim A., Frey B. (2004) Early stage litter decomposition rates for Swiss forests. Biogeochemistry, 70, 299–313, doi:10.1007/s10533-003-0844-5.

Heim A., Wehrli L., Eugster W., Schmidt M. W. I. (2009) Effects of sampling design on the probability to detect soil carbon stock changes at the Swiss Carbo Europe site Lägeren. Geoderma, 149, 347–354, doi:10.1016/j.geoderma.2008.12.018.

IUSS Working Group WRB (2007) World reference base for soil resources 2006. World Soil Resources Reports No. 103, FAO, Rome.

Jansson P. E., Karlberg L. (2001) Coupled heat and mass transfer model for soil-plant-atmosphere systems. ftp://www.lwr.kth.se/CoupModel/CoupModel.pdf. Accessed 22 September 2009.

Joos O., Hagedorn F., Heim A., Gilgen A. K., Schmidt M. W. I., Siegwolf R. T. W., Buchmann N. (2010) Summer drought reduces total and litter-derived soil CO_2 effluxes in temperate grassland – clues from a ^{13}C litter addition experiment. Biogeosciences, 7, 1031–1041, doi:10.5194/bg-7-1031-2010.

Kaiser K., Guggenberger G. (2000) The role of DOM sorption to mineral surfaces in the preservation of organic matter in soils. Organic Geochemistry, 31, 711–725, doi:10.1016/S0146-6380(00)00046-2.

Kaiser K., Guggenberger G. (2007) Distribution of hydrous aluminium and iron over density fractions depends on organic matter load and ultrasonic dispersion. Geoderma 140, 140–146, doi:10.1016/j.geoderma.2007.03.018.

Kalbitz K., Schmerwitz J., Schwesig D., Matzner E. (2003) Biodegradation of soil-derived dissolved organic matter as related to its properties. Geoderma, 113, 273–291, doi:10.1016/S0016-7061(02)00365-8

Kalbitz K., Kaiser K. (2008) Contribution of dissolved organic matter to carbon storage in forest mineral soils. Journal of Plant Nutrition and Soil Science, 171, 52–60, doi:10.1002/ jpln.200700043.

Kramer C., Trumbore S., Fröberg M., Cisneros Dozal L. M., Zhang D., Xu X., Santos G. M., Hanson P. J. (2010) Recent (<4 year old) leaf litter is not a major source of microbial carbon in a temperate forest mineral soil. Soil Biology and Biochemistry, 42, 1028–1037 doi:10.1016/j.soilbio.2010.02.021.

Kuzyakov Y., Friedel J. K., Stahr K. (2000) Review of mechanisms and quantification of priming effects. Soil Biology and Biochemistry, 32, 1485–1498, doi:10.1016/S0038-0717(00)00084-5.

LeMellec A., Meesenburg H., Michalzik B. (2010) The importance of canopy-derived dissolved and particulate organic matter (DOM and POM) – comparing throughfall solution from broadleaved and coniferous forests. Annals of Forest Science, 67, doi:10.1051/forest/2009130.

Liski J., Nissinen A., Erhard M., Taskinen O. (2003) Climatic effects on litter decomposition from arctic tundra to tropical rainforest. Global Change Biology, 9, 1–10, doi:10.1046/j.1365-2486.2003.00605.x.

Liski J., Palosuo T., Peltoniemi M., Sievänen R. (2005) Carbon and decomposition model Yasso for forest soils. Ecological Modelling, 189, 168–182, doi:10.1016/j.ecolmodel.2005.03.005.

Magill A. H., Aber J. D. (2000) Dissolved organic carbon and nitrogen relationships in forest litter as affected by nitrogen deposition. Soil Biology and Biochemistry, 32, 603–613, doi:10.1016/S0038-0717(99)00187-X.

Moore T. R., Trofymow J. A., Taylor B., Prescott C., Camiré C., Duschene L., Fyles J., Kozak L., Kranabetter M., Morrison I., Siltanen M., Smith S., Titus B., Visser S., Wein R., Zoltai S. (1999) Litter decomposition rates in Canadian forests. Global Change Biology, 5, 75–82, doi:10.1046/j.1365-2486.1998.00224.x.

Müller M., Alewell C., Hagedorn F. (2009) Effective retention of litter-derived dissolved organic carbon in organic layers. Soil Biology and Biochemistry, 41(6), 1066–1074, doi:10.1016/j.soilbio.2009.02.007.

Neff J. C., Asner G. P. (2001) Dissolved organic carbon in terrestrial ecosystems: Synthesis and a Model. Ecosystems 4, 29–48, doi:10.1007/s100210000058.

Ngao J., Epron D., Brechet C., Granier A. (2005) Estimating the contribution of leaf litter decomposition to soil CO_2 efflux in a beech forest using ^{13}C-depleted litter. Global Change Biology, 11, 1768–1776, doi: 10.1111/j.1365-2486.2004.01014.x.

Nottingham A. T., Griffiths H., Chamberlain P. M., Stott A. W., Tanner E. V. J. (2009) Soil priming by sugar and leaf-litter substrates: A link to microbial groups. Applied Soil Ecology, 42, 183–190, doi:10.1016/j.apsoil.2009.03.003.

Ohlsson K. E. A., Bhupinderpal S., Holm S., Nordgren A., Lovdahl L., Högberg P. (2005) Uncertainties in static closed chamber measurements of the carbon isotopic ratio of soil-respired CO_2. Soil Biology and Biochemistry, 37, 2273–2276.

Park J. H., Kalbitz K., Matzner E. (2002) Resource control on the production of dissolved organic carbon and nitrogen in a deciduous forest floor. Soil Biology and Biochemistry, 34, 813–822, doi:10.1016/S0038-0717(02)00011-1.

Park J. H., Matzner E. (2003) Controls on the release of dissolved organic carbon and nitrogen from a deciduous forest floor investigated by manipulations of aboveground litter inputs and water flux. Biogeochemistry, 66, 265–286, doi:10.1023/B:BIOG.0000005341.19412.7b.

Pinheiro J., Bates D., DebRoy S., Sarkar D., and The R Core Team (2008) nlme: Linear and Nonlinear Mixed Effects Models. R package version 3. 1–89.

Potter C. S., Klooster S. A. (1997) Global model estimates of carbon and nitrogen storage in litter and soil pools: response to changes in vegetation quality and biomass allocation. Tellus, 49(1), 1–17.

Rey A., Pegoraro E., Tedeschi V., De Parri I., Jarvis P. G., Valentini R. (2002) Annual variation in soil respiration and its components in a coppice oak forest in Central Italy. Global Change Biology, 8, 851–866, doi:10.1046/j.1365-2486.2002.00521.x.

Rochette P., Angers D. A., Flanagan L. B. (1999) Maize residue decomposition measurement using soil surface carbon dioxide fluxes and natural abundance of carbon-13. Soil Science Society of America Journal, 63, 1385–1396.

Roscoe R., Buurman P., Velthorst E. J. (2000) Disruption of soil aggregate by different amounts of ultrasonic energy in SOM fractionation of a clay Latosol: carbon, nitrogen, and $\delta^{13}C$ distribution in particle-size fractions. European Journal of Soil Science, 51, 445–454.

Rubino M., Dungait J. A. J., Evershed R. P., Bertolini T., De Angelis P., D'Onofrio A., Lagomarsino A., Lubritto C., Merola A., Terrasi F., Cotrufo M. F. (2010) Carbon input belowground is the major C flux contributing to leaf litter mass loss: Evidences from a ^{13}C labelled-leaf litter experiment. Soil Biology and Biochemistry, 42, 1009–1016, doi:10.1016/j.soilbio.2010.02.018.

Ruehr N. K., Knohl A., Buchmann N. (2010) Environmental variables controlling soil respiration on diurnal, seasonal and annual time-scales in a mixed mountain forest in Switzerland. Biogeochemistry, 98, 153–170, doi:10.1007/s10533-009-9383-z.

Santruckova H., Bird M. I., Lloyd J. (2000) Microbial processes and carbon-isotope fractionation in tropical and temperate grassland soils. Functional Ecology, 14, 108–114, doi:10.1046/j.1365-2435.2000.00402.x.

Schaefer M., Migge-Kleian S., Scheu S. (2009) The role of soil fauna for decomposition of plant residues. In: Functioning and management of European beech ecosystems, edited by: Brumme R., Khanna P. K., Ecological Studies, 208, Springer, Berlin, 207–230, doi:10.1007/b82392_13.

Scheu S. (1997) Effects of litter (beech and stinging nettle) and earthworms (Octolasion lacteum) on carbon and nutrient cycling in beech forests on a basalt-limestone gradient: A laboratory experiment. Biology and Fertility of Soils, 24, 384–393, doi:10.1007/s003740050262.

Schweizer M., Fear J., Cadish G. (1999) Isotopic (^{13}C) fractionation during plant residue decomposition and its implications for soil organic matter studies. Rapid Communication in Mass Spectrometry, 13, 1284–1290.

Six J., Conant R. T., Paul E. A., Paustian K. (2002) Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. Plant Soil, 241, 155–176, doi:10.1023/A:1016125726789.

Six J., Frey S. D., Thiet R. K., Batten K. M. (2006) Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Science Society of America Journal, 70, 555–569, doi:10.2136/sssaj2004.0347.

Subke J. A., Hahn V., Battipaglia G., Linder S., Buchmann N., Cotrufo M. F. (2004) Feedback interactions between needle litter decomposition and rhizosphere activity. Oecologia 139:551–559. doi:10.1007/s00442-004-1540-4.

Sulzman E. W., Brant J. B., Bowden R. D., Lajtha K. (2005) Contribution of aboveground litter, belowground litter, and rhizosphere respiration to total soil CO_2 efflux in an old growth coniferous forest. Biogeochemistry, 73, 231–256, doi: 10.1007/s10533-004-7314-6.

Swanston C. W., Torn M. S., Hanson P. J., Southon J. R., Garten C. T., Hanlon E. M., Ganio L. (2005) Initial characterization of processes of soil carbon stabilization using forest stand-level radiocarbon enrichment. Geoderma, 128, 52–62, doi:10.1016/j.geoderma.2004.12.015.

Tipping, E. (2002) Cation binding by humic substances, University Press, Cambridge, UK.

Vesterdal L (1997) Influence of soil type on mass loss and nutrient release from decomposing foliage litter of beech and Norway spruce. Canadian Journal of Forest Research, 29, 95–105.

Walthert L., Zimmermann S., Blaser P., Luster J., Lüscher P. (2004) Waldböden der Schweiz. Band 1: Grundlagen und Region Jura, Hep Verlag, Bern.

Walthert L., Graf U., Kammer A., Luster J., Pezzotta D., Zimmermann S., Hagedorn F. (2010) Determination of organic and inorganic carbon, $\delta^{13}C$, and nitrogen in soils containing carbonates after acid fumigation with HCl. Journal of Plant Nutrition and Soil Science, 173, 207–216, doi:10.1002/jpln.200900158.

Wu J., Joergensen R. G., Pommerening B., Chaussod R., Brookes P. C. (1990) Measurement of soil microbial biomass C by fumigation-extraction–an automated procedure. Soil Biology and Biochemistry, 22, 1167–1169.

Zeller B., Colin-Belgrand M., Dambrine E., Martin F., Bottner P. (2000) Decomposition of ^{15}N-labelled beech litter and fate of nitrogen derived from litter in a beech forest. Oecologia, 123, 550–559, doi:10.1007/PL00008860.

Paper II

Mineralisation, leaching and stabilisation of ^{13}C-labelled leaf and twig litter in a beech forest soil

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Abstract

Very few field studies have quantified the different pathways of C loss from decomposing litter even though this is essential to understand long-term dynamics of C stocks in soils. Using ^{13}C-labelled leaf (isotope ratio ($\delta^{13}C$) = –40.8‰) and twig litter ($\delta^{13}C$ = –38.4‰), we tracked the litter-derived C into soil respiration, into dissolved organic C (DOC) and into soil organic matter of a beech forest in the Swiss Jura mountains. After one year of decomposition, mass loss in the litter layer was almost twice as great for leaves as it was for twigs (75% vs. 40%). This difference was not the result of a slow mineralisation of the woody litter, but primarily of the only slight incorporation of twig-derived C into mineral soils. The C mineralisation rates of the twig litter were only slightly lower than those of the leaf litter (10–35%), particularly after the loss of the readily available litter fraction. However, the leaching of DOC from twigs amounted only to half of that from leaves. Tracing the litter-derived DOC showed that DOC from both litter types was mostly retained (88–96%) and stabilised in the top centimetres of the mineral soil. In the soil organic C at 0–2 cm depth, we recovered 8% of the initial leaf C, but only 4% of the twig C. Moreover, the ^{13}C mass balance suggested that a substantial fraction of the leaf material (~ 30%) was transported via soil fauna to soil depths below 2 cm, while the twig litter mainly decomposed *in situ* on the soil surface, probably due to its rigid structure and low nutritional value. In summary, our study shows that decaying twigs are rapidly mineralised, but are less important for the C storage in this beech forest soils than leaf litter.

1. Introduction

Litterfall represents the mayor nutrient flux in temperate forests and often accounts for more than half of the annual C input to soils (Meentemeyer et al., 1982; Perruchoud et al., 1999). How much the aboveground litter contributes to the soil C pool in the long term depends considerably on the rate at which its C is either mineralised to CO_2 or incorporated into mineral soils through soil fauna and dissolved organic C (DOC) (Rubino et al., 2010).

Decay rates of litter are related to climatic conditions (Liski et al., 2003), but they can also vary significantly between litter materials at the same forest site (Moore et al., 1999). Here, C/N ratios and lignin concentrations have often been found to be the best predictor of C losses from litter (e.g. Heim & Frey, 2004; Hagedorn & Machwitz, 2007). Ligneous tissues of twigs with low N contents are, therefore, supposed to be much more resistant to microbial decay than leaf litter, even though different kinds of fungi have proved to be very effective in the degradation of woody tissues (Griffith & Body, 1991).

Based on this mechanistic concept, most soil C models assume clearly slower decay and transformation rates for twig than for leaf litter (Liski et al., 2005; Carrasco et al., 2006; Scott et al., 2006). However, only a few studies have compared the decomposition pathways of twigs and leaves in the field, even though fine woody litter contributes about 30% to annual litterfall in temperate forests (Thürig et al., 2005). Litterbag studies in China and along a climatic gradient in Finland found that leaf and needle litter lost about twice as much C as twig litter (Guo et al., 2007; Vávřová et al., 2009). By contrast, very small differences in C losses from litterbags were observed between beech leaves and spruce branchlets on a Rendzina soil in Switzerland (Hättenschwiler et al., 1999).

Particularly little is known about the translocation of twig-derived C to mineral soils. For instance, we are not aware of any study that has measured DOC leaching from decomposing twigs in the field. Leaching of DOC from leaf litter can contribute to 10–30% of total C losses from litter (Magill & Aber, 2000; Hagedorn & Machwitz, 2007), and might be important for the C transport to mineral soils, where it is either immobilized by microbes or adsorbed on mineral surfaces (Kalbitz & Kaiser, 2008). Incubation studies suggest that, after the loss of the water-soluble fraction, DOC leached from litter derives predominantly from degradation products of lignin (Kalbitz et al., 2006). Consequently, lignin-rich litter such as twigs should have a particularly high potential to release DOC in later stages of decomposition. Several studies have indeed observed enhanced DOC fluxes below decaying coarse woody debris (Yano et al., 2005; Zalamea et al., 2007; Kahl, 2008). Moreover, more twig-derived DOC might be retained in mineral soils than leaf-derived DOC since the high-molecular-weight, lignin-derived components of DOC, the so-called ‘hydrophobic’ DOC fractions have a higher affinity to mineral surfaces than the ‘hydrophilic’ fractions with fewer functional groups (Kaiser & Guggenberger, 2000).

In the last decade, several studies have taken advantage of isotopically labelled litter to investigate not only the mass loss but also the pathways of decomposition of leaf, needle and root litter (e.g. Bird & Torn, 2006; Fröberg et al., 2009; Rubino et al., 2010). Isotopic labels allow the estimation of litter contributions to soil respiration as well as the tracking of litter-derived C from the forest floor to mineral soils. We have found, however, no study which has applied this powerful approach to assess C fluxes from decomposing twig litter. Thus, the fate of twig-derived C is still very uncertain: is it mainly respired back to the atmosphere or does it contribute significantly to the long-term storage of C in forest soils?

The aim of this study was to compare the decomposition pathways of leaf and twig litter in a mixed beech forest in the Swiss Jura mountains. Over the course of one year, we

measured CO_2 production, DOC leaching and translocation of C from ^{13}C-depleted leaves and twigs originating from four-year-old beech trees. The specific objectives of our study were: (1) to test the general assumption that fine woody litter decomposes much more slowly than non-woody litter; (2) to assess the contribution of decaying twigs and leaves to soil respiration and DOC fluxes in forest soils; and (3) to estimate how much of the leaf and twig litter is incorporated into mineral soils, and thus might contribute to the long-term storage of C in calcareous forest soils.

2. Materials and Methods

2.1 Study site description

The experimental site is in a mixed beech forest on the relatively steep (on average 24°) south-facing slope of the Lägeren mountain (680 m a.s.l.). This hill range is situated about 20 km NW of Zurich (47°28'40.8'' N, 8°21'55.2'' E) and belongs to the easternmost part of the Jura mountain range. As a contribution to the CarboEurope IP, net ecosystem CO_2 exchange and soil respiration have been measured routinely there for several years (Etzold et al., 2010; Ruehr et al., 2010). The mean annual temperature is 8.4°C and the mean annual precipitation is 930 mm. The litter experiment was carried out on two soil types 200 m apart and with different parent materials. One of the soils is a Rendzic Leptosol (Rendzina) overlying limestone debris and the other a Haplic Cambisol on a bedrock of marl. The properties of the topsoils (0–10 cm) are presented in Table 1. Both soils have mull-type organic layers indicative of a high level of biological activity, but the pH and soil organic C content of the topsoils are higher in the Rendzina than in the Cambisol. The overstory vegetation is more diverse on the Rendzina where, in addition to beech (*Fagus sylvatica* L.) and spruce trees (*Picea abies* (L.) Karst.) growing on both soils, also ash (*Fraxinus excelsior* L.) and maple trees (*Acer pseudoplatanus* L.) occur. The annual litterfall is larger on the Rendzina (330 g C m^{-2}) than on the Cambisol (230 g C m^{-2}), but consists of about 70% leaf litter and of 30% fine woody litter in both soils (N. Ruehr, personal communication).

Table 1. *Properties of the top 0–10 cm of soil. Five soil cores (5 cm in diameter) were taken from both soil types. The values are means ± standard errors.*

	pH ($CaCl_2$)	Particle-size distribution (%)			Fine-earth bulk density (g cm^{-3})	C_{org} (%)	C/N	C_{org} pool (kg m^{-2})	$\delta^{13}C_{org}$ (‰)
		250–2000 µm	2–250 µm	< 2 µm					
Rendzina	7.5 (0.1)	25 (2)	21 (3)	54 (5)	0.91 (0.03)	3.9 (0.3)	12.0 (0.1)	3.6 (0.2)	–27.2 (0.2)
Cambisol	5.9 (0.1)	23 (4)	35 (2)	42 (3)	0.94 (0.6)	2.8 (0.5)	11.3 (0.5)	2.6 (0.1)	–26.7 (0.2)

2.2 Labelled litter experiment

The litter experiment started in November 2007, lasted for one year and included three different litter treatments. In plots of 50 × 50 cm, the native litter layer was replaced either through ^{13}C-labelled beech leaves (isotope ratio ($\delta^{13}C$) = –40.8‰; referred to as 'soil + leaves'), ^{13}C-labelled twigs ($\delta^{13}C$ = –38.4‰; 'soil + twigs') or polystyrene shreds ('bare soil'). The later was used to mimic a litter layer and its impact on soil moisture and temperature. To recover the isotopic label for both litter types equally well, we added larger amounts of twigs (2 kg m^{-2}) than of leaves (0.75 kg m^{-2}) since the woody litter was expected to decompose much more slowly. The labelled litter originated from the final harvest of beech saplings from a four-year CO_2 enrichment experiment in Switzerland that used ^{13}C-depleted CO_2 (Hagedorn et al., 2005). The twigs had diameters ranging from 1 to 8 mm (4 mm on average), and were cut into pieces 4 to 8 cm in length.

In both soils, each litter treatment was replicated five times. The replicates were arranged in five groups within a radius of 10 m, each consisting of the three different treatments. The distance between the litter plots within a group was about 1 m. To prevent litter loss due to wind and inputs of fresh litter, the litter plots were framed with acrylic glass (12 cm height) and covered with a polyethylene net (mesh size = 0.7 × 0.3 mm). We also minimized root respiration by digging a 30 cm deep trench around each plot to amplify the ^{13}C signal of litter-derived CO_2. A plastic foliar was inserted to prevent external root ingrowths. Vegetation growth on the plots was suppressed by periodically weeding.

2.3 Soil respiration and its $\delta^{13}C$

Soil CO_2 effluxes were measured with a portable infrared gas analyzer (Li-8100, LI-COR Inc., Lincoln, NE, USA) at bi-weekly intervals between October 2007 (one month before litter addition) and November 2008. The chamber of the IRGA was placed on permanently installed PVC collars (5 cm high, 20 cm in diameter), inserted into the soils to a depth of 2 cm.

To estimate the contribution of litter-derived CO_2, the ^{13}C signature of the soil respiration ($\delta^{13}C_{resp}$) was determined with the closed soil chamber method on ten sampling dates (e.g. Ohlsson et al., 2005). Depending on the CO_2-efflux, the soil collars were closed for 8–40 min with a lid, allowing for a CO_2 increase of about 400 ppm. At the end of the accumulation period, one gas sample was taken from each chamber with a syringe through a septum in the lid and injected into glass vials (12 ml), which had been previously evacuated and closed with an airtight rubber septum. In addition, gas samples were collected next to each collar

immediately after they had been closed (ambient air). The gas samples were analysed for both the CO_2 concentration and the $\delta^{13}C$ using a Gasbench II, coupled with a isotope ratio mass spectrometer Delta Plus (both Thermo Finnigan Mat, Bremen, Germany). More details on the IRMS system employed in this study can be found in Joos et al. (2008).

To correct for the contamination of chamber CO_2 with ambient CO_2, $\delta^{13}C_{resp}$ was calculated with the following mixing model:

$$\delta^{13}C_{resp} = (\delta^{13}C_{chamber} \times [CO_2]_{chamber} - \delta^{13}C_{ambient} \times [CO_2]_{ambient}) / ([CO_2]_{chamber} - [CO_2]_{ambient}), \quad (1)$$

where $[CO_2]$ is the concentration and $\delta^{13}C$ the isotopic composition of CO_2 in the ambient air and in the soil chamber.

2.4 Water, litter and soil sampling

Water was sampled 1.5 m above the forest floor (throughfall) with PE funnels (Ø 11 cm), below the litter with zero-tension lysimeters (13 × 17 cm PVC boxes) and at a soil depth of 5 cm with suction plates (Ø 5.5 cm) made of borosilicate glass (pore size P5; Schmizo, Zofingen, Switzerland). Four openings (Ø 1cm) on the bottom of the zero-tension lysimeters allowed soil animals to feed on the litter. The soil solution in the suction plates was evacuated by applying a constant low pressure of –400 hPa with a vacuum pump (EcoTech, Bonn, Germany). Both the lysimeters and the suction plates were installed on the lower side of the litter plots. The soil water was continuously collected in 0.5 L bottles, which were buried in the soil and emptied after every larger rain event.

At the start of the experiment, a small part of the added litter (2.5 g of leaf litter and 10 g of twig litter) was placed in litterbags (10 × 10 cm; polypropylene) with mesh sizes of 1 mm. After one year, the bags were collected from the forest floor and the litter that remained was cleaned to remove mineral particles, dried at 60°C for chemical analysis and at 105°C to determine the dry mass. The same procedure was applied to the unconfined labelled litter that remained on the surface. Subsequently, a soil core (Ø 5 cm) 10 cm in length was taken from each plot, frozen and divided into 2 cm thick layers with a hacksaw. The soil samples were freed from roots, dried at 60°C and sieved (< 2mm).

2.5 Chemical analysis

All water samples were passed through 0.45-µm cellulose-acetate filters (Schleicher & Schuell, ME25), pooled on a monthly base and refrigerated until analysis. To remove

inorganic C, HCl suprapur (30%) was added to all samples. DOC concentrations were determined with a TOC/TN analyzer (TOC-V, Shimadzu Corporation, Tokyo, Japan). The molar UV absorptivity at 285 nm in the DOC was measured using a Cary 50 UV-spectrophotometer (Varian, Palo Alto, USA). Aliquots of 50–80 ml were freeze-dried to determine the $\delta^{13}C$ of the DOC. To facilitate the weighing of the freeze-dried dissolved organic matter, 5 mg of K_2SO_4 was added to each sample.

The concentrations and the isotope ratios of C and N in litter, soil and freeze-dried samples were measured with an elemental analyzer (Euro EA 3000, HEKAtech, Germany) coupled to an isotope ratio mass spectrometer (Delta V Advantage, Thermo, Germany). Both the fresh and the decomposed litter were additionally analysed for: (1) hot water extractables by extracting 1 g of milled sample three times with 25 ml of hot (85 ± 5°C) water and once with cold water (15 min each); (2) phenolics by applying the Folin-Denis colorimetric method to the water extracts (Swain & Hillis, 1959); (3) Klason lignin. The Klason lignin was the residue of milled litter after it had been extracted with hot water and ethanol, hydrolyzed with 3 ml of 72% sulphuric acid for 1 h at 30°C and, after addition of 84 ml water, autoclaved for 1 h at 120°C. (4) The soluble lignin was estimated from the UV absorbance of the hydrolisate at 205 nm (Dence, 1992).

The microbial biomass in the litter layer was analysed 4 and 12 months after litter addition using the chloroform-fumigation extraction approach (Brooks et al., 1985). Briefly, 5 g of litter was fumigated for 24 h with $CHCl_3$ and then extracted with 50 ml of 0.25 *M* K_2SO_4. The microbial C and N were calculated from the differences in the C and N concentrations between these extracts and additional extracts from non-fumigated samples, assuming extraction efficiencies of 0.45 (K_{EC}) and 0.54 (K_{EN}) (Jensen et al., 1997).

2.6 Meteorological measurements

Thermocouples connected to the portable IRGA were used to measure the temperatures in the air, in the litter layer and at soil depths of 5 cm and 10 cm for each sampling location at the same time as the measurements of the CO_2 effluxes. In addition, soil temperatures were recorded continuously with temperature loggers (ibuttons, Maxim Integrated Products DS1922L, USA) installed in three replicates per treatment at a soil depth of 10 cm. Moreover, a meteo station 100 m away from the experimental site recorded air temperature, soil moisture at depths of 5, 10, 30 and 50 cm, air humidity, wind speed and net radiation, all with intervals of 30 min. Precipitation was measured at an eddy covariance flux tower 80 m away.

2.7 Calculations and statistics

Litter-derived C: The contribution of labelled litter C (f_{litter}) to soil-C fluxes and pools was calculated for each plot individually as follows:

$$f_{litter} = (\delta^{13}C_{soil+litter} - \delta^{13}C_{control}) / \Delta^{13}C, \quad (2)$$

where $\delta^{13}C_{soil+litter}$ is the $\delta^{13}C$ measured in the 'soil + litter' treatment, $\delta^{13}C_{control}$ is the ^{13}C signature in the adjacent 'bare soil' plot and $\Delta^{13}C$ is the difference in the $\delta^{13}C$ between the bulk litter (–38.4‰ and –40.8‰) and the soil organic C (SOC; –26.7 to –27.8‰). This approach is based on the assumption that isotopic fractionation of ^{13}C was minimal, or at least the same, in the litter layer and the mineral soil during both C mineralisation and DOC production (e.g. Schweizer et al., 1999; Santruckova et al., 2000; Fröberg et al., 2007).

DOC fluxes: The vertical fluxes of DOC below the litter layer and at a depth of 5 cm were estimated by multiplying the DOC concentrations by water fluxes simulated with the COUP model (Jansson & Karlberg, 2001). The model was parameterised using the organic C content, the particle-size distribution of different soil layers and several other parameters. The input variables were air temperature, precipitation, vapour pressure, wind speed and net radiation. Finally, soil moisture data were used to validate the model.

Modelling CO_2 effluxes: The temperature dependency of the soil-respired CO_2 was fitted with the following equation (see Fang & Moncrieff, 2001):

$$CO_{2\,soil} = a \times (T - T_{min})^b, \quad (3)$$

where T is the soil temperature at a depth of 10 cm, and T_{min}, a, and b are parameters derived from non-parametric curve fits (Origin 7.1, OriginLab, USA).

However, it was not possible to fit the litter-derived CO_2 effluxes to a simple temperature function since the litter C pool declines with time. For modelling the C respired from the added litter, we thus used the temperature dependency of CO_2 effluxes in the 'bare soil' treatment and scaled this function to the litter-derived C effluxes at the beginning of January by linear transformation:

$$CO_{2\,litter} = a \times (T - T_{min})^b \times S, \quad (4)$$

where the transformation factor S is the theoretical ratio of litter-derived CO_2 and mineral soil-derived CO_2 at identical soil and air temperatures. We selected the values in January as a reference because litter contributed most to the soil respiration on this sampling date. In a next

step, the mineralisation potential of litter C was expressed as the ratio between measured and theoretical (no change in C pool) litter-derived CO_2 fluxes, which were calculated with Eq. 4 for all sampling days. This ratio (factor *P*) was used as a correction factor:

$$CO_{2\ litter} = a \times (T - T_{min})^b \times S \times P. \quad (5)$$

To estimate the daily C losses from the litter through CO_2 release, we interpolated *P* between the sampling days and used the air temperature as input variable.

Statistics: Differences in C fluxes and C pools between the litter treatments were tested with linear mixed effect models using the nlme package from R version 2.8.1 (Pinheiro et al., 2008). By including random effects for the 'plot group' and for each single 'litter plot', the models accounted for both the split unit design of the experiment and the repeated measurement structure. Beside the litter type, soil and time were used as fixed factors. In all final models, normality and homoscedasticity of the residuals were verified visually with diagnostic plots and, when necessary, the dependent variable was log transformed.

3. Results

3.1 Changes in the mass and quality of the litter

The amount of litter C that remained in litterbags after one year of decomposition ranged from 66 to 73% (Fig. 1). It was larger on the Rendzina than on the Cambisol ($p<0.01$), and was slightly but not significantly larger for twig than for leaf litter (69.5% vs. 67.5%; $p = 0.19$). In contrast, the proportion of the ^{13}C-depleted litter recovered in the litter layer (not confined in litterbags) was twice (Cambisol) and three times (Rendzina) as large for twig litter (57–61%) as it was for leaf litter (23–31%; Fig. 1).

Table 2. *Selected parameters of leaf and twig litter at the beginning and after 1 year of decomposition. The values are means ± standard errors.*

Litter	Time	C / N	Hot water-soluble substances (mg / g)	Fraction of phenols in water solubles (%)	Lignin[a] (mg / g)	$\delta^{13}C_{org}$ (‰)	Microbial C / Microbial N
Leaf	fresh	28 (1)	247 (7)	25 (1)	340 (9)	–40.8 (0.2)	11 (0.3)
Twig	"	95 (2)	127 (5)	10 (0)	280 (2)	–38.4 (0.1)	14 (0.3)
Leaf	1 year	20 (1)	66 (4)	10 (1)	530 (8)	–40.4 (0.2)	6.5 (0.4)
Twig	"	51 (6)	72 (8)	6 (1)	420 (20)	–38.3 (0.5)	12 (0.5)

[a] Klason lignin + soluble lignin

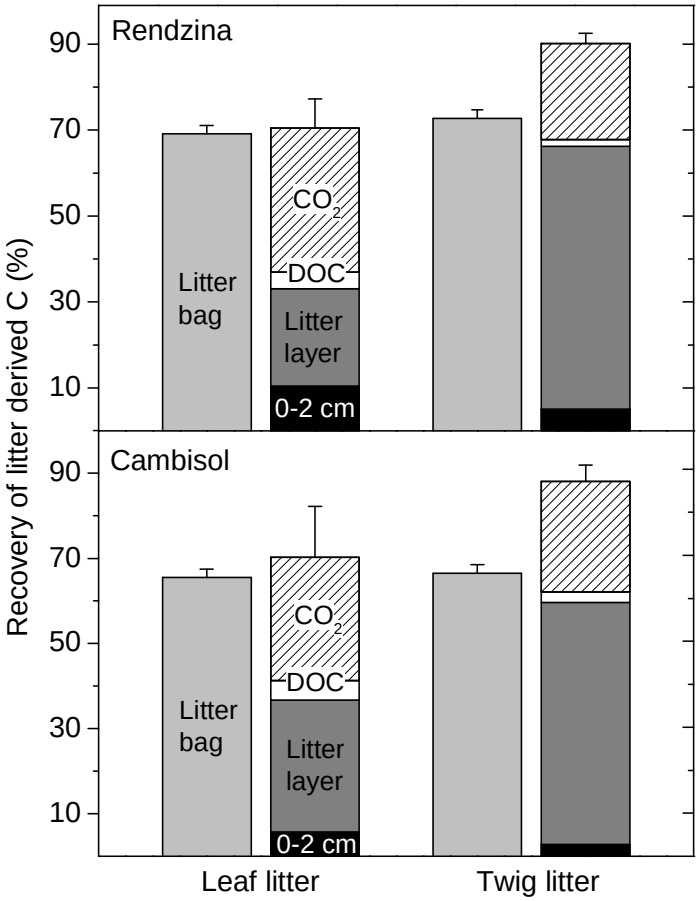

Figure 1. *Total recovery of the ^{13}C-labelled litter C in litterbags and in different C fluxes and C pools after 1 year of decomposition. Means and standard errors of five replicates.*

The C/N ratio of both the bulk litter and the microbial biomass on the litter decreased over the course of the experiment and was clearly wider in the twig than in the leaf litter (Table 2). After one year, concentrations of hot water-soluble substances were equally small in both litter types, whereas this fraction was twice as much in the leaf as in the twig litter at the beginning. The lignin concentrations (Klason lignin + soluble lignin) increased by a factor of 1.5 during decomposition, and, surprisingly, were about 20% lower in the twig than in the leaf litter (Table 2). Only a slight (+0.1–0.4‰) and not significant increase in the δ^{13}C of the litter material was observed.

3.2 Contribution of litter C to SOC

At the end of the experiment, slight shifts (0.2–0.5‰; $p < 0.01$) in the δ^{13}C indicated that recent litter C contributed to 2–5% of the C pools at 0–2 cm depth, corresponding to about 4% of the initial twig C and to about 8% of the initial leaf C (Fig. 1). However, no significant litter effect on the δ^{13}C of SOC was observed at depths below 2 cm.

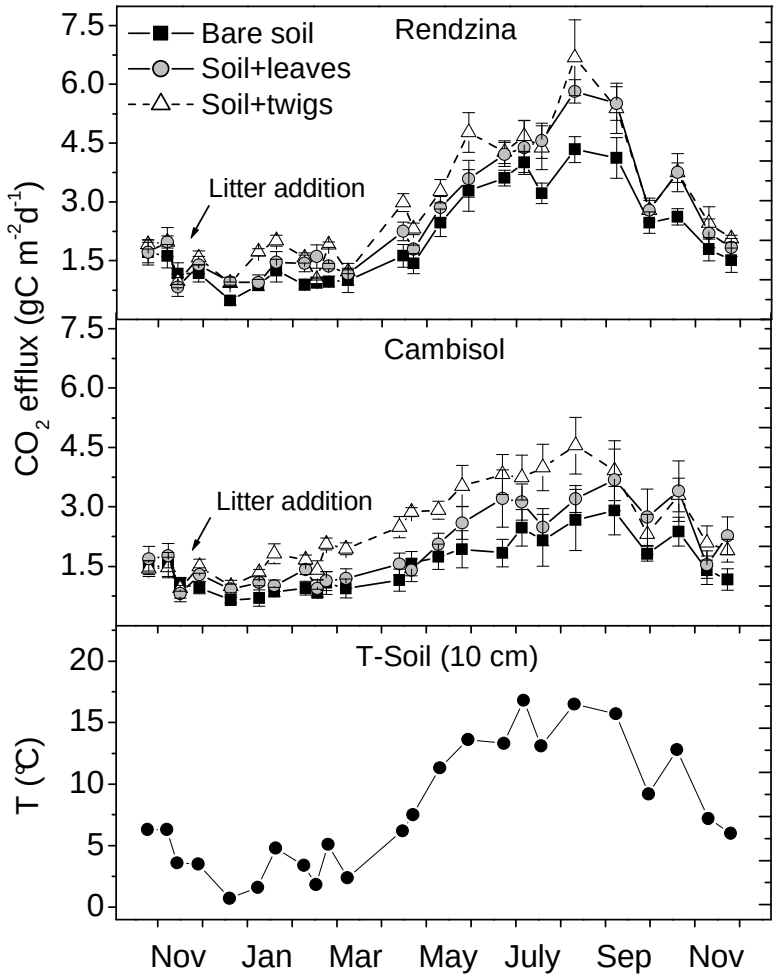

Figure 2. *Seasonal course of the heterotrophic soil respiration in the Rendzina and the Cambisol and of the soil temperature at a depth of 10 cm. The CO_2 effluxes are the means of five replicates (±standard error).*

3.3 CO_2 effluxes

The addition of leaf litter (0.75 kg m^{-2}) and twig litter (2 kg m^{-2}) to bare soils had distinct positive effects on soil CO_2 effluxes throughout the experiment ($p < 0.001$; Fig. 2). Moreover, the CO_2 release was significantly larger in plots with twig litter than in those with leaf litter (+25%; $p < 0.001$). Using the strong dependency of the soil respiration on the temperature at a depth of 10 cm ($R^2 = 0.85–0.97$; Eq. 3), we estimated that total C losses from the soils ranged from 575 g m^{-2} yr^{-1} in the bare Cambisol to 1038 g m^{-2} yr^{-1} in the Rendzina with a twig layer (Table 3).

Table 3. *Total C loss from forest soils through CO_2 and cumulated DOC fluxes below the litter layer and in the mineral soil at a depth of 5 cm during the course of the litter experiment (November 2007–2008). The values are the means of five replicates (± standard errors).*

Soil	Treatment	CO_2 release (gCO_2-C m^{-2})	DOC litter layer (gDOC m^{-2})	DOC at 5 cm (gDOC m^{-2})
Rendzina	Bare soil	803 (71)	-	8.9 (1.8)
	+ Leaves (0.75 kg m^{-2})	973 (52)	20.4 (3.5)	11.7 (1.2)
	+ Twigs (2 kg m^{-2})	1038 (59)	21.8 (4.6)	12.4 (1.3)
Cambisol	Bare soil	575 (106)	-	9.2 (1.9)
	+ Leaves (0.75 kg m^{-2})	683 (128)	21.5 (1.8)	8.5 (1.5)
	+ Twigs (2 kg m^{-2})	888 (94)	29.1 (4.7)	9.8 (1.4)

The ^{13}C signature of CO_2 respired from the bare soils varied between –23 and –28‰ (Fig. 3) in both the Rendzina and the slightly acidic Cambisol. Small differences in the $\delta^{13}C$ between the two soils indicate that the dissolution of carbonates was a negligible source of CO_2 in the Rendzina. The decomposition of ^{13}C-depleted leaves ($\Delta^{13}C$= –13.6‰) and twigs ($\Delta^{13}C$= –11.2 ‰) decreased the $\delta^{13}C$ of soil CO_2 effluxes on average by 4.5‰ in winter and by 2.5‰ over the warm season (Fig. 3).

The fraction of litter-derived C in the soil CO_2 effluxes (f_{litter}) peaked at 45–60% in January shortly after a cold period during which no litter decomposition was observed at air temperatures clearly below 0°C (Fig. 4). While f_{litter} in the 'soil + leaves' plots declined continuously with increasing time of decomposition to about 10% at the end of the experiment, no significant time effect on f_{litter} was found in the 'soil + twigs' plots from February to November ($p = 0.13$). As a consequence, f_{litter} was not dependent on the litter type in winter ($p = 0.79$), but was considerably larger for twigs than for leaves over the warm season independent of the soil type ($p < 0.001$). In agreement with this temporal pattern, twig-derived C was mineralised 40% more slowly than leaf-derived C in winter, but only slightly and not significantly more slowly over the warm season (–15%; $p = 0.31$). By modelling CO_2 effluxes from litter between measurements (Eq. 5), we estimated that, after one year, the twig litter had lost 22–26% of its initial C through CO_2 and the leaf litter 29–34% (Fig. 5).

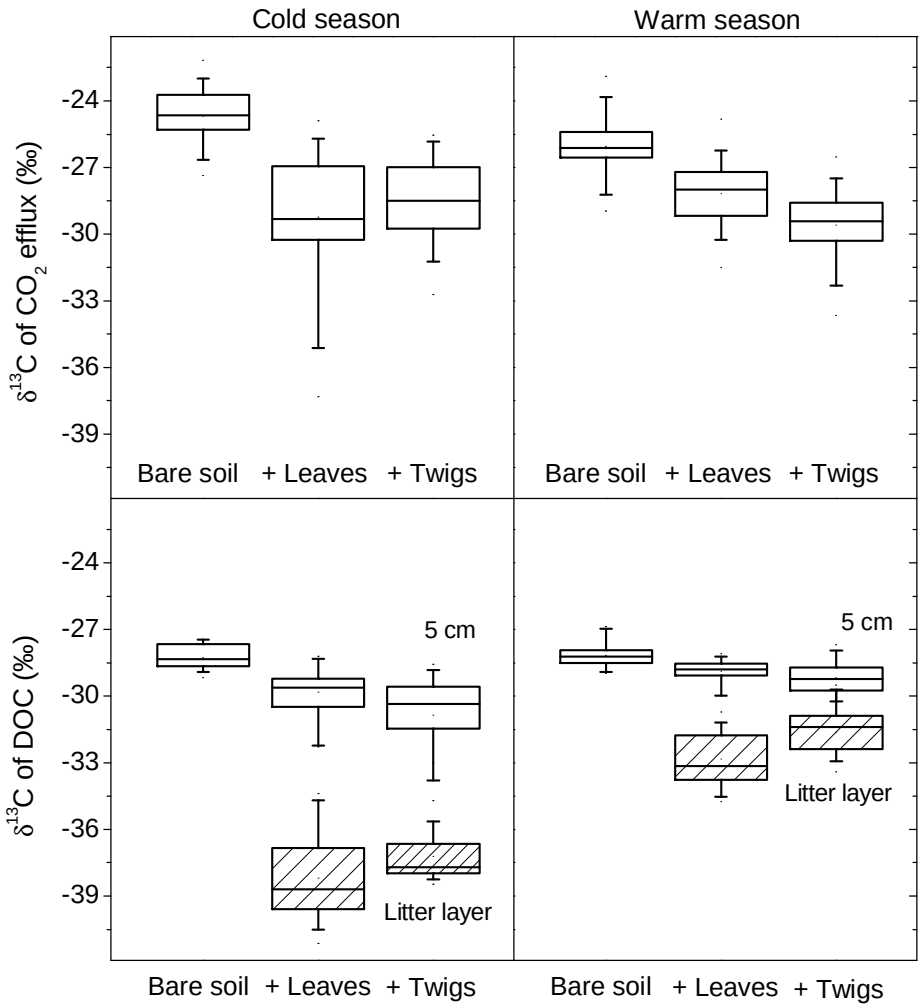

Figure 3. *Variability in the $\delta^{13}C$ of the soil CO_2 efflux (upper figures) and of the DOC leached from both the litter layer and the mineral soil at a depth of 5 cm. The values of the Rendzina and the Cambisol are combined. Each box shows the median value, the quartiles and the 2.5%- and 97.5%-quantiles of 50 single measurements for the CO_2 and of 30 measurements for the DOC in the cold (November 07–April 08) and in the warm season (April 08–November 08).*

3.4 DOC fluxes

The total fluxes of DOC decreased from 20–29 g DOC m^{-2} yr^{-1} below the litter layer to 9–12.5 g DOC m^{-2} yr^{-1} at a soil depth of 5 cm, with only marginal differences between the twig and leaf litter treatments, as well as between the two soil types (Table 3). The ^{13}C tracing revealed that litter-derived C contributed to, on average, 70% of the DOC leached from the litter layer but to only 11% of the DOC leached from mineral soils (Figs. 3 and 4). Therefore, litter-derived DOC was mostly retained (88–96%) in the top centimetres of the soil profile and most of the DOC at a depth of 5 cm originated from 'older' SOM.

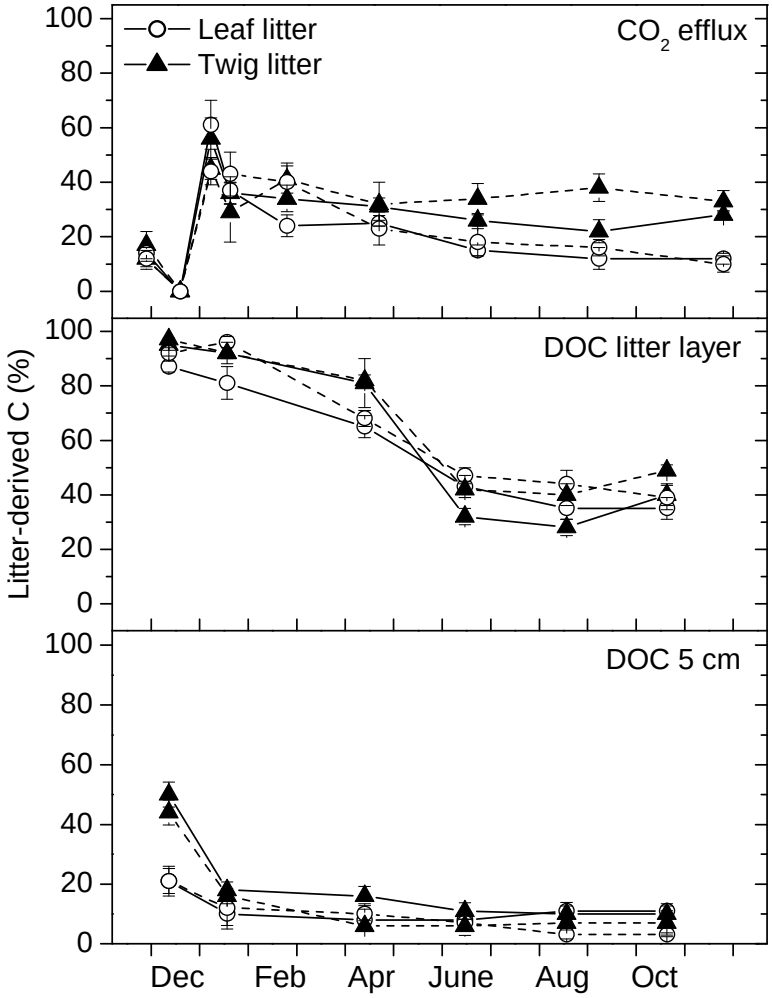

Figure 4. *Contribution of litter-derived C to the heterotrophic soil respiration and to the DOC leached from the litter layer and from the mineral soil at a depth of 5 cm. Means and standard errors of five replicates in the Rendzina (solid line) and the Cambisol (dashed line).*

The seasonal dynamics of litter-derived DOC were very similar for both litter types. An initial flush of DOC from the litter layer, associated with heavy rainfalls in early winter, was followed by clearly lower and constant leaching rates throughout the rest of the experiment (Fig. 5). The leaching rates, however, were much lower for twig than for leaf litter during both the initial DOC flush and the subsequent leaching cycles ($p < 0.001$). Over one year, the twig litter lost 1.5–2.5% of its initial C pool through leaching of DOC, whereas the leaf litter lost 4–5% of its C through this pathway. Thus, the DOC leaching corresponded to about 8% and 13% of the C respired as CO_2 from the twig and leaf litter, respectively.

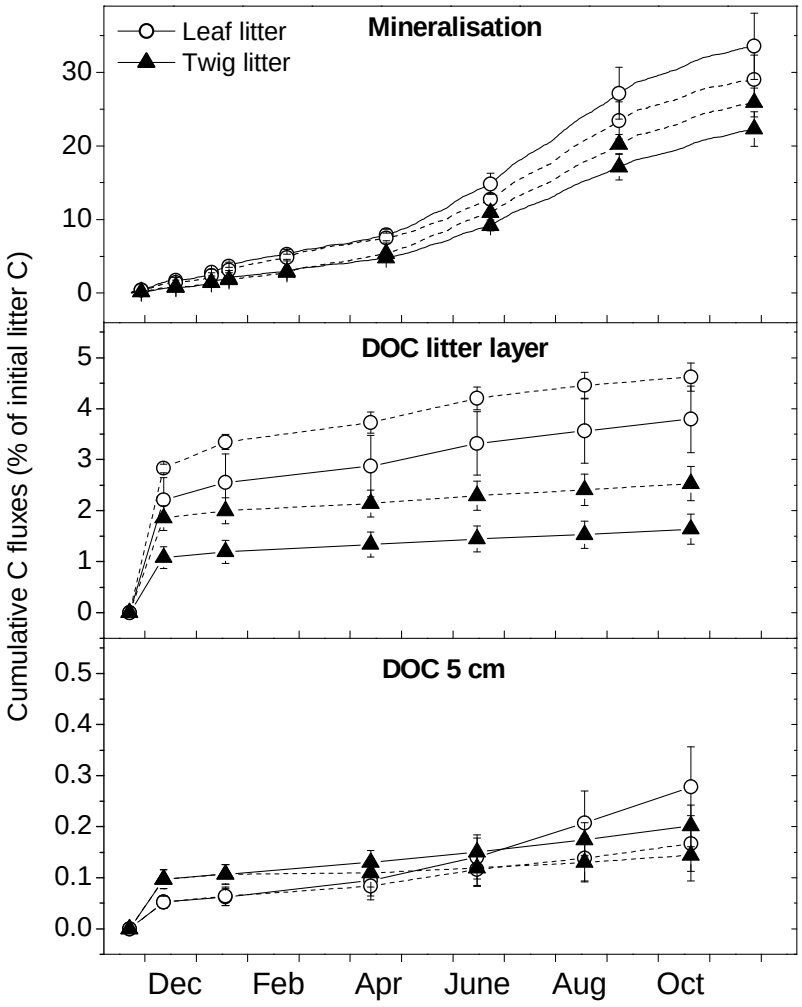

Figure 5. *Seasonal dynamics of litter-derived C respired as CO_2, leached as DOC from the litter layer and recovered in the DOC at a depth of 5 cm. The solid line represents the Rendzina and the dashed line the Cambisol. All values are the means of five replicates (± standard error).*

In contrast to the DOC leaching below the litter layer, the amount of litter-derived DOC detected in mineral soils was not significantly lower for twig than for leaf litter (–20%; $p = 0.12$; Fig. 4). Consequently, less DOC leached from twigs was retained when it passed through the uppermost mineral soil than DOC leached from leaves. Furthermore, less litter-derived DOC was recovered in the mineral soils of the Cambisol than in those of the Rendzina (–40%; $p < 0.05$).

We assessed the quality of litter-derived DOC using the UV absorbance at 285 nm of soil water, which was corrected for throughfall DOC with a simple mixing model. The correction was necessary since throughfall DOC had a clearly lower UV absorptivity than the litter-derived DOC (on average 200 vs. 300 L mol^{-1} cm^{-1}), and contributed large amounts to the

DOC leached from the litter layer especially after the green up of trees in spring (Fig. 4). This is indicated by the $\delta^{13}C$ of the DOC (Fig. 3). The UV absorptivity of litter DOC greatly increased during the course of the experiment and peaked in summer at 350–450 L mol^{-1} cm^{-1} (Fig. 6). The twig-derived DOC also had a lower UV absorptivity (–15%) than the leaf-derived DOC throughout the experiment ($p < 0.001$).

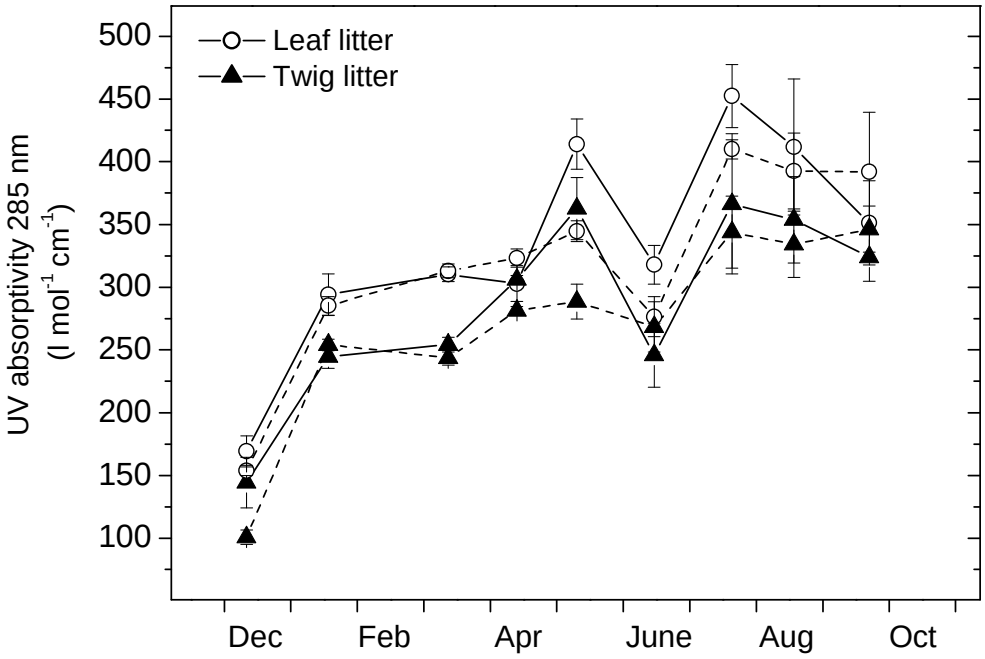

Figure 6. *Molar UV absorptivity of litter-derived C leached from the forest floor in the Rendzina (solid line) and the Cambisol (dashed line). Means and standard errors of five replicates.*

4. Discussion

4.1 Almost equal mineralisation of ^{13}C-labelled leaf and twig litter

Fine woody litter is commonly thought to decompose much more slowly than leaf litter (Liski et al., 2005). The recovery of the ^{13}C-labelled litter on the soil surface (unconfined) appears to confirm this assumption. One year after litter addition, about 60% of the twig litter C remained in the litter layer, more than twice as much as that of the leaf litter (Fig. 1).

Our results show, however, that microbial decomposition was not the main reason for the different mass losses from leaves and twigs in the forest floor. Contrary to our expectations, the mineralisation rates of the two litter types differed surprisingly little. Cumulated over one year, the twigs lost only 10–35% less C through CO_2 than the leaves (Fig. 5). In the Cambisol,

the rates at which the two litter types mineralised even became equal after the loss of the most labile C pool at the end of winter. In agreement with the C mineralisation rates of the ^{13}C-labelled litter, the twigs in the litterbags lost only slightly, but not significantly, less C than the confined leaves (Fig. 1). Our findings are supported by a study with litterbags (mesh-sizes of 0.02–2 mm) on a Rendzina soil near Basel (Switzerland), where the mass losses after one year of decomposition were very similar for beech leaves and spruce branchlets (Hättenschwiler et al., 1999). Almost identical mineralisation rates for both litter types were also found in a lab experiment using a mixture of beech and oak litter (Park et al., 2002). In our study, the differences between the litter types were less pronounced in litterbags than in the unconfined ^{13}C-labelled litter (Fig. 1), possibly because the mesh bags inhibited the fragmentation of the leaf litter through soil macrofauna, and thus suppressed litter decay (Cotrufo et al., 2010). In contrast to the leaf litter, twig litter was not fragmented either inside or outside the litterbags.

The small differences we found between the mineralisation rates of leaf and twig litter can probably be attributed to both a relatively fast decomposition of beech twigs and a relatively slow decomposition of beech leaves because: (1) the annual C losses from twigs through CO_2 and DOC observed in our study (24–33%) were at the upper end of weight losses (15–31%) found across several forest ecosystems and tree species of the temperate zone (Boddy & Swift, 1984); (2) C losses from beech leaves determined in litterbags and laboratory experiments are commonly among the lowest of various leaf litter types (Moore et al., 1999; Hoorens et al., 2003; Hagedorn & Machwitz, 2007) possibly because they are tough, have a comparatively small proportion of water solubles and are rich in lignin and polyphenols (Schaefer et al., 2009). Therefore, we assume that similar decay rates for fine woody and non-woody litter is a specific phenomena for beech, while in forest ecosystems dominated by other tree species, the decomposition of the two litter types might differ much more. Large differences between leaves and twigs have recently been observed, for instance, for litter from *Tilia*, *Betula*, *Picea* and *Pinus* (Guo et al., 2007; Vávřová et al., 2009).

Surprisingly, the measurement of the Klason lignin indicated a smaller proportion of lignin in twig than in leaf litter (Table 2), which seems to be in conflict with the woody tissue of the twigs. However, it is known that the Klason procedure can overestimate lignin in plant tissues that contain other high-molecular-weight components, such as proteins and tannins (Hammel, 1997). We assume that the beech leaves contained a significant fraction of these interfering substances. The evidence that both litter types were rich in refractory components fits our finding of similar C mineralisation rates. This agreement, in turn, suggests that the

decomposability of these two litter types was controlled primarily by the fraction of high-molecular-weight substances, and less importantly by the initial N concentration, which was four times lower in the twig litter (Table 2). Finally, it should be noted that the diameters of the twigs used in this experiment were relatively small (0.1–0.8 mm) and hence, the bark-to-wood ratio was high. This ratio might be positively correlated with the decomposability of twigs and branches as the bark is more enriched in nutrients than the wood, and larger diameters impede the access of the microbes to the inner parts of woody litter (Swift, 1977; Miller, 1983).

One aim of this study was to assess how much decomposition of recent litter (< 1 yr) contributes to soil CO_2 effluxes at the Lägeren research site. Our results show that in these beech forest ecosystem litter-derived CO_2 is a major component (~ 50%) of soil CO_2 effluxes mainly on warm winter days when the leaf litter is still fresh (Fig. 4). On an annual scale, however, decaying leaf litter accounted only for 10–12% of the annual C losses from soils through CO_2 and twig litter for 4–6%. This was estimated by combining the rates of the C mineralisation (220–340 mg CO_2-C g litter C^{-1} yr^{-1}) with the amounts of litterfall at our site. The fraction of leaf litter is roughly half of that found in a ^{13}C-tracer study in a French beech forest. In contrast to our study, they estimated twice as large C losses through CO_2 from leaf litter (62% of initial C during one year), possibly because they interpolated between the litter-derived CO_2 effluxes and did not account for the temperature dependency of litter decomposition.

4.2 Twig litter is a small source of DOC

Several studies of coarse woody debris have suggested that DOC leached from decaying wood is a significant transport pathway of C from forest floors to mineral soils (Zalamea et al., 2007; Kahl, 2008). Our results, however, provide little evidence that this applies also to decaying twigs in beech forests. Leaching of DOC from twig litter amounted to only half of that from leaf litter throughout the experiment, which contrasts with similar C mineralisation rates of the two litter types (Fig. 5). These findings are supported by an incubation experiment with forest floor material from a German beech forest, where the net release of DOC differed much more between leaf and fine woody litter than the CO_2 production (Park et al., 2002). We think that the reduced leaching of twig-derived DOC resulted in part from the limited contact of the inner parts of the twigs with the percolating water and hence from the spatial segregation of a substantial proportion of the woody material from the leaching.

Interestingly, DOC leached from the twigs was lower in refractory components, and hence probably more biodegradable than leaf-derived DOC. This was indicated by the smaller molar UV absorbance of the twig litter DOC (Fig. 6), which suggests smaller proportions of aromatic compounds and a higher biodegradability of the DOC (Dilling & Kaiser, 2002; Hagedorn & Machwitz, 2007). The UV absorbance of litter-derived DOC was lower for twigs than for leaves not only during the initial DOC flush, which probably consisted largely of water-soluble substances in the litter itself (Fröberg et al., 2007), but also thereafter, when DOC is assumed to be generated during the degradation of lignin (Kalbitz et al., 2006). This finding corresponds with analyses of DOC leached from eight different types of leaf litter, which showed that the biodegradability of DOC was negatively correlated to the decomposability of the litter material (Hagedorn & Machwitz, 2007). Moreover, our results are in agreement with the litter manipulation experiment at the DIRT study site in Oregon, in which DOC derived from recent coarse woody debris contained a slightly larger hydrophilic fraction than DOC leached from the litter layer (Yano et al., 2005).

The reason for the leaching of more biodegradable DOC from the woody litter could be a different microbial community on the two litter types. The C/N ratio of the microbial biomass was clearly higher for twigs than for leaves (Table 2), which suggests that fungi are more dominant on the woody litter (Ross & Sparling, 1993). Fungi are better adapted to degrading lignin-derived C (Hammel, 1997). Thus, aromatic compounds in the twig litter might be mineralised more completely than in the leaf litter. This could also have contributed to the small net release of DOC from the twigs as compared to the C mineralisation.

By tracking the ^{13}C-signal of litter-derived DOC in the mineral soil, we found that less than 10% of the DOC leached from the litter layer was recovered in DOC at a depth of 5 cm, and the greatest fraction of litter DOC was thus retained in the uppermost mineral soil. This strong immobilisation of forest floor DOC confirms results from the long-term litter manipulation at the Oregon DIRT site, where the DOC mass balance indicated that DOC from coarse woody litter was largely removed with its passage across the organic layers and mineral soils (Yano et al., 2005). Similar retentions of DOC have recently been observed for ^{13}C- and ^{14}C-labelled leaf and needle litter (Fröberg et al., 2007 and 2009; Müller et al., 2009).

Our results suggest that that sorption of DOC to mineral surfaces was the key mechanism for the retention of litter DOC: (1) DOC was strongly immobilised in winter and thus at low microbial activities. (2) Litter-derived DOC was retained more effectively in the more acidic Cambisol than in the Rendzina, possibly due to a stronger sorption to soil minerals at lower

pH values (Tipping, 2002). (3) Moreover, the retention of litter-derived DOC in the mineral soil was stronger for twigs than for leaves (Fig. 4). The most likely reason for this is that twig-derived DOC had a lower specific UV absorbance (Fig. 6), and thus contained less 'hydrophobic' DOC, which has a higher affinity to mineral surfaces than 'hydrophilic' DOC (Kaiser & Guggenberger, 2000).

In summary, the tracing of litter-derived DOC showed that less DOC was leached from twigs than from leaves and that the twig DOC was less strongly retained in the mineral soil. Both findings suggest that the sorptive stabilisation of litter-derived C via leaching is less important for twig than for leaf litter. This is further confirmed by the recovery of labelled litter C in the SOC at 0–2 cm depth, where 8% of the initial leaf C was stored at the end of the experiment in contrast with only 4% of the twig C (Fig. 6). A substantial source of this 'new' SOC was probably DOC leached from the litter layer.

4.3 Biologically mediated transport of litter

We have strong evidence that the export of litter via soil fauna played an important role primarily for the leaf litter, even though this pathway of C loss was not explicitly measured. In both soils, the sum of C fluxes from the ^{13}C-depleted litter and the litter recovered on the soil surface and at a depth of 0–2 cm amounted to about 90% of the added twig litter C, but only to 70% of the initial leaf litter C (Fig. 1). We assume that the missing C in this mass balance can be attributed to a biologically mediated transport of litter-derived C to the deeper soil, where it was no longer detectable as the ^{13}C label vanished in the large SOC pool.

Our estimation that 30% of the leaf-derived C were translocated via faunal activity is similar to findings from a recent tracer experiment in an Italian poplar forest (Rubino et al., 2010) and a microcosm experiment in calcareous soils (Scheu, 1997), where soil fauna removed 30–60% of the leaf litter during one year. The ^{13}C-mass balance in our study additionally indicates that the proportion of twig litter that was incorporated into mineral soils by bioturbation was about 10%, and thus only one third of the leaf litter. These estimates are confirmed by the mass losses from the litterbags with a mesh size of 1 mm, which excludes macro fauna. After one year, about twice as much leaf litter remained in the litterbags as in the unconfined litter on the forest floor (Fig. 1). In contrast, litterbags only slightly affected the mass loss from the twig litter. This finding is in accordance with that of Hättenschwiler et al. (1999) that restricting access of soil fauna to decomposing litter affected mass losses from beech leaves but not from spruce branchlets.

Implications for C storage in forest soils

One of the uncertainties in predicting future C stocks in forest soils is the relative contribution of different types of litter to SOM (Crow et al., 2009). Our ^{13}C-tracer experiment in a temperate beech forest suggests that decomposing twigs are less important for the C storage in these soils than leaves because: (1) The C mineralisation rates of the two litter types differed little (10–35%), in particular after the loss of the readily available litter fraction. By multiplying the rates of C loss through CO_2 with the annual litterfall, to which leaves contribute 70% and twigs 'only' 30%, we estimated that the net input of C to the soil after one year of decomposition is approximately twice as large for leaf as for twig litter. (2) The twig litter also appears to have a considerably lower potential to be transferred and stabilised in the mineral soils via organo-mineral interactions than the leaf litter. Much less of the twig-derived C was transported to mineral soils over one year than of the leaf-derived C through DOC leaching or through bioturbation. Moreover, the DOC leached from twigs probably had a lower affinity to mineral surfaces than leaf DOC as it contained less 'hydrophobic' components. More twig litter will probably not be transported downwards until twigs lose their rigid structure and break down into smaller pieces. By that stage of decomposition, however, a large proportion of twig C might have already been mineralised to CO_2, and thus would not contribute to C storage in mineral soils.

Our findings go against the assumption of most soil C models (e.g. YASSO), which basically assume that fine woody litter mineralises much more slowly than leaf litter, but that similar proportions of the decomposed litter are transferred into more stable humus pools (Liski et al., 2005; Carrasco et al., 2006; Scott et al., 2006). While the first assumption may possibly apply to litter from many tree species other than beech (Guo et al., 2007; Vávřová et al., 2009), we propose that the ratio of mineralisation and incorporation into mineral soil C is distinctly larger for twig litter than for leaf litter in most forest ecosystems of the temperate zone. More tracer studies, however, are needed to confirm this conclusion.

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References

Bird J. A., Torn M. S. (2006) Fine roots vs. needles: a comparison of ^{13}C and ^{15}N dynamics in a ponderosa pine forest soil. Biogeochemistry, 79, 361–382, doi:10.1007/s10533-005-5632-y.

Boddy L., Swift M. J. (1984) Wood decomposition in an abandoned beech and oak coppiced woodland in SE England: III. Decomposition and turnover of twigs and branches, Holarctic Ecology., 7, 229–238.

Brookes P. C., Landman A., Pruden G., Jenkinson D. S. (1985) Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass in soil. Soil Biology and Biochemistry, 17, 837–842.

Carrasco J. J., Neff J. C., Harden J. W. (2006) Modeling physical and biogeochemical controls over carbon accumulation in a boreal forest soil. Journal of Geophysical Research, 111, G02004, doi: 10.1029/2005JG000087.

Cotrufo M. F., Ngao J., Marzaioli F., Piermatteo D. (2010) Inter-comparison of methods for quantifying above-ground leaf litter decomposition rates. Plant Soil, 334, 365–376, doi:10.1007/s11104-010-0388-0.

Crow S. E., Lajtha K., Filley T. R., Swanston, C. W., Bowden R. D., Caldwell B. A. (2009) Sources of plant-derived carbon and stability of organic matter in soil: implications for global change. Global Change Biology, 15, 2003–2019, doi: 10.1111/j.1365-2486.2009.01850.x.

Dence C. W. (1992) The determination of lignin. In: Methods in lignin chemistry, edited by: Lin, S. Y. and Dence, C.W., Springer, Berlin, 33–61.

Dilling J., Kaiser, K. (2002) Estimation of the hydrophobic fraction of dissolved organic matter in water samples using UV photometry. Water Research, 36, 5037–5044, doi:10.1016/S0043-1354(02)00365-2.

Etzold S., Buchmann N., Eugster W. (2010) Contribution of advection to the carbon budget measured by eddy covariance at a steep mountain slope forest in Switzerland. Biogeosciences, 7, 2461–2475, doi:10.5194/bg-7-2461-2010.

Fang C., Moncrieff J. B. (2001) The dependence of soil CO_2 efflux on temperature. Soil Biology and Biochemistry, 33, 155–165, doi:10.1016/S0038-0717(00)00125-5.

Fröberg M., Berggren Kleja D., Hagedorn F. (2007) The contribution of fresh litter to dissolved organic carbon leached from a coniferous forest floor. European Journal of Soil Science, 58, 108–114, doi:10.1111/j.1365-2389.2006.00812.x.

Fröberg M., Hanson P. J., Trumbore S. E., Swanston C. W., Todd D. E. (2009) Flux of carbon from ^{14}C-enriched leaf litter throughout a forest soil mesocosm. Geoderma, 149, 181–188, doi:10.1016/j.geoderma.2008.11.029.

Griffith G. S., Boddy L. (1991) Fungal decomposition of attached angiosperm twigs. III. Effect of water potential and temperature on fungal growth, survival and decay of wood. New Phytologist, 117, 259–269.

Guo Z., Li Q., Zheng J., Liu W., Fan C., Ma Y., Yu G., Han S. (2007) Leaf and twig litter decomposition of main species in different forests along the north slope of Changbai Mountain, northeast China. Frontiers of Forestry in China, 2, 47–54, doi 10.1007/s11461-007-0007-2.

Hagedorn F., Maurer S., Bucher J. B., Siegwolf R. T. W. (2005) Immobilization, stabilization and remobilization of nitrogen in forest soils at elevated CO_2: a ^{15}N and ^{13}C tracer study. Global Change Biology, 11, 1816–1827, doi:10.1111/j.1365-2486.2005.01041.x.

Hagedorn F., Machwitz M. (2007) Controls on dissolved organic matter leaching from forest litter grown under elevated atmospheric CO_2. Soil Biology and Biochemistry, 39, 1759–1769, doi:10.1016/j.soilbio.2007.01.038.

Hammel K. E. (1997) Fungal degradation of lignin. In: Driven by nature: Plant litter quality and decomposition, edited by: Cadish, G., Giller, K. E., CAB International, Wallingford, UK, 33–45.

Hättenschwiler S., Bühler S., Körner C. (1999) Quality, decomposition and isopod consumption of tree litter produced under elevated CO_2. Oikos, 85, 271–281.

Heim A., Frey B. (2004) Early stage litter decomposition rates for Swiss forests. Biogeochemistry, 70, 299–313, doi:10.1007/s10533-003-0844-5.

Hoorens B., Aerts R., Stroetenga M. (2003) Does initial litter chemistry explain litter mixture effects on decomposition?. Oecologia, 137, 578–586, doi:10.1007/s00442-003-1365-6.

Jansson P. E., Karlberg L. (2001) Coupled heat and mass transfer model for soil-plant-atmosphere systems. ftp://www.lwr.kth.se/CoupModel/CoupModel.pdf. Accessed 22 September 2009.

Jensen L. S., Müller T., Magid J., Nielsen N. E. (1997) Temporal variation of C and N mineralisation, microbial biomass and extractable organic pools in soil after oilseed rape straw incorporation in the field. Soil Biology and Biochemistry, 29, 1043–1055.

Joos O., Saurer M., Heim A., Hagedorn F., Schmidt M. W. I., Siegwolf, R. T. W. (2008) Can we use the CO_2 concentrations determined by continuous-flow isotope ratio mass spectrometry from small samples for the Keeling plot approach?. Rapid Communication in Mass Spectrometry, 22, 4029–4034.

Kahl, T. (2008) Kohlenstofftransport aus dem Totholz in den Boden. Ph.D. thesis, University of Freiburg i. Brsg., Germany, 121 pp., avalaible at http://www.freidok.uni-freiburg.de/volltexte/5522/.

Kaiser K., Guggenberger G. (2000) The role of DOM sorption to mineral surfaces in the preservation of organic matter in soils. Organic Geochemistry, 31, 711–725, doi:10.1016/S0146-6380(00)00046-2.

Kalbitz K., Kaiser K., Bargholz J., Dardenne P. (2006) Lignin degradation controls the production of dissolved organic matter in decomposing foliar litter. European Journal of Soil Science, 57, 504–516, doi: 10.1111/j.1365-2389.2006.00797.x.

Kalbitz K., Kaiser K. (2008) Contribution of dissolved organic matter to carbon storage in forest mineral soils. Journal of Plant Nutrition and Soil Science, 171, 52–60, doi:10.1002/ jpln.200700043.

Liski J., Nissinen A., Erhard M., Taskinen O. (2003) Climatic effects on litter decomposition from arctic tundra to tropical rainforest. Global Change Biology, 9, 1–10, doi:10.1046/j.1365-2486.2003.00605.x.

Liski J., Palosuo T., Peltoniemi M., Sievänen R. (2005) Carbon and decomposition model Yasso for forest soils. Ecological Modelling, 189, 168–182, doi:10.1016/j.ecolmodel.2005.03.005.

Magill A. H., Aber J. D. (2000) Dissolved organic carbon and nitrogen relationships in forest litter as affected by nitrogen deposition. Soil Biology and Biochemistry, 32, 603–613, doi:10.1016/S0038-0717(99)00187-X.

Meentemeyer V., Box E. O., Thompson R. (1982) World patterns and amounts of terrestrial plant litter production. BioScience, 32, 125–128.

Miller, W. E. (1983) Decomposition rates of aspen bole and branch litter. Forest Science, 29, 351–356.

Moore T. R., Trofymow J. A., Taylor B., Prescott C., Camiré C., Duschene L., Fyles J., Kozak L., Kranabetter M., Morrison I., Siltanen M., Smith S., Titus B., Visser S., Wein R., Zoltai S. (1999) Litter decomposition rates in Canadian forests. Global Change Biology, 5, 75–82, doi:10.1046/j.1365-2486.1998.00224.x.

Müller M., Alewell C., Hagedorn F. (2009) Effective retention of litter-derived dissolved organic carbon in organic layers. Soil Biology and Biochemistry, 41, 1066–1074, doi:10.1016/j.soilbio.2009.02.007.

Ngao J., Epron D., Brechet C., Granier A. (2005) Estimating the contribution of leaf litter decomposition to soil CO_2 efflux in a beech forest using ^{13}C-depleted litter. Global Change Biology, 11, 1768–1776, doi: 10.1111/j.1365-2486.2004.01014.x.

Ohlsson K. E. A., Bhupinderpal S., Holm S., Nordgren A., Lovdahl L., Högberg P. (2005) Uncertainties in static closed chamber measurements of the carbon isotopic ratio of soil-respired CO_2. Soil Biology and Biochemistry, 37, 2273–2276.

Park J. H., Kalbitz K., Matzner E. (2002) Resource control on the production of dissolved organic carbon and nitrogen in a deciduous forest floor. Soil Biology and Biochemistry, 34, 813–822, doi:10.1016/S0038-0717(02)00011-1.

Perruchoud D., Kienast F., Kaufmann E., Bräker O. U. (1999) 20th century carbon budget of forest soils in the Alps. Ecosystems, 2, 320–337, 1999.

Pinheiro J., Bates D., DebRoy S., Sarkar D., and The R Core Team (2008) nlme: Linear and Nonlinear Mixed Effects Models. R package version 3. 1–89.

Ross D. J., Sparling G. P. (1993) Comparison of methods to estimate microbial C and N in forest floor and soil under Pinus radiate on a coastal sand. Soil Biology and Biochemistry, 25, 1591–1599.

Rubino M., Dungait J. A. J., Evershed R. P., Bertolini T., De Angelis P., D'Onofrio A., Lagomarsino A., Lubritto C., Merola A., Terrasi F., Cotrufo M. F. (2010) Carbon input belowground is the major C flux contributing to leaf litter mass loss: Evidences from a ^{13}C labelled-leaf litter experiment. Soil Biology and Biochemistry, 42, 1009–1016, doi:10.1016/j.soilbio.2010.02.018.

Ruehr N. K., Knohl A., Buchmann N. (2010) Environmental variables controlling soil respiration on diurnal, seasonal and annual time-scales in a mixed mountain forest in Switzerland. Biogeochemistry, 98, 153–170, doi:10.1007/s10533-009-9383-z.

Santruckova H., Bird M. I., Lloyd J. (2000) Microbial processes and carbon-isotope fractionation in tropical and temperate grassland soils. Functional Ecology, 14, 108–114, doi:10.1046/j.1365-2435.2000.00402.x.

Schaefer M., Migge-Kleian S., Scheu S. (2009) The role of soil fauna for decomposition of plant residues. In: Functioning and management of European beech ecosystems, edited by: Brumme R., Khanna P. K., Ecological Studies, 208, Springer, Berlin, 207–230, doi:10.1007/b82392_13.

Scheu S. (1997) Effects of litter (beech and stinging nettle) and earthworms (Octolasion lacteum) on carbon and nutrient cycling in beech forests on a basalt-limestone gradient: A laboratory experiment. Biology and Fertility of Soils, 24, 384–393, doi:10.1007/s003740050262.

Schweizer M., Fear J., Cadish G. (1999) Isotopic (^{13}C) fractionation during plant residue decomposition and its implications for soil organic matter studies. Rapid Communication in Mass Spectrometry, 13, 1284–1290.

Scott N. A., Tate K. R., Ross D. J., Parshotam A. (2006) Processes influencing soil carbon storage following afforestation of pasture with Pinus radiata at different stocking densities in New Zealand. Australian Journal of Soil Research, 44, 85–96.

Swain T., Hillis W. E. (1959) The phenolic constituents of Prunus domestica. I. The quantitative analysis of phenolic constituents. Journal of the Science of Food and Agriculture, 10, 63–68.

Swift M. J. (1977) The ecology of wood decomposition. Science Progress, 64, 175–199.

Thürig E., Palosuo T., Bucher J., Kaufmann, E. (2005) The impact of windthrow on carbon sequestration in Switzerland: a model-based assessment. Forest Ecology and Management, 210, 337–350, doi:10.1016/j.foreco.2005.02.030.

Tipping, E. (2002) Cation binding by humic substances. University Press, Cambridge, UK.

Vávřová P., Penttila T., Laiho, R. (2009) Decomposition of Scots pine fine woody debris in boreal conditions: implications for estimating carbon pools and fluxes. Forest Ecology and Management, 257, 401–412, doi:10.1016/j.foreco.2008.09.017.

Yano Y., Lajtha K., Sollins P., Caldwell B. A. (2005) Chemistry and dynamics of dissolved organic matter in a temperate coniferous forest on Andic soils: effects of litter quality. Ecosystems, 8, 286–300, doi: 10.1007/s10021-005-0022-9.

Zalamea M., González G., Ping C. L., Michaelson, G. (2007) Soil organic matter dynamics under decaying wood in a subtropical wet forest: effect of tree species and decay stage. Plant Soil, 296, 173–185, doi: 10.1007/s11104-007-9307-4.

Paper III

Application of a quantum cascade laser-based spectrometer in a closed chamber system for real-time $\delta^{13}C$ and $\delta^{18}O$ measurements of soil-respired CO_2

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Abstract

Laser spectrometry is an emerging technique to analyse the stable isotopic composition of soil-respired CO_2 ($\delta^{13}C_{resp}$, $\delta^{18}O_{resp}$) *in situ* and at high temporal resolution. Here we present the first application of a quantum cascade laser-based spectrometer (QCLS) in a closed soil-chamber system to determine simultaneously $\delta^{13}C_{resp}$ and $\delta^{18}O_{resp}$. In a Swiss beech forest, a total of 90 chamber measurements with 20 min sampling time each were performed. The instrument measured the $\delta^{13}C$ and $\delta^{18}O$ of the CO_2 in the chamber headspace at every second with a precision of 0.25‰, resulting in Keeling plots with 1200 data points. In addition, we calculated $\delta^{13}C_{resp}$ directly from the flux ratio of $^{13}CO_2$ and $^{12}CO_2$. The flux-ratio values were 0.8‰ lower than the Keeling plot intercepts when the flux rates were derived from quadratic curve fits of the CO_2 increase. The $\delta^{18}O$-Keeling plots showed a significant bending very likely due to the equilibration of chamber CO_2 with the ^{18}O of surface soil water. Therefore, we used a quadratic curve fit of the Keeling plots to estimate $\delta^{18}O_{resp}$. Our results also revealed that $\delta^{13}C_{resp}$ was not constant throughout the CO_2 accumulation in the closed soil chambers: There were significant but non-systematic variations in $\delta^{13}C_{resp}$ for the first ten minutes, and systematic shifts in $\delta^{13}C_{resp}$ of on average 1.9‰ in the second part of the 20-min measurements. These biases were probably caused by non-steady-state conditions in the soil-chamber system. Our study illustrates that the high temporal resolution of QCLS measurements allows the detection of non-linearities in the isotopic effluxes of CO_2 from the soil due to soil-chamber feedbacks. This information can be used to improve the estimates for $\delta^{13}C_{resp}$ and $\delta^{18}O_{resp}$.

Keywords: Soil respiration, Stable isotopes, Laser spectroscop, Closed soil chamber, Keeling plot; ^{13}C labelled litter

1. Introduction

Modelling soil respiration requires the partitioning of the soil-CO_2 efflux into its autotrophic and heterotrophic components, which have individual responses to environmental changes (Boone et al., 1998). The use of the stable isotopes ^{13}C and ^{18}O as tracers is an excellent way to partition soil respiration components because it allows the source of the released CO_2 to be identified with minimal disturbance of the soil environment (Ehleringer et al., 2000; Hanson et al., 2000; Kuzyakov, 2006). Moreover, the natural abundance of ^{13}C in soil-CO_2 effluxes

can provide a direct link to assimilation processes in the plant system (Ekblad & Högberg, 2001).

The stable-isotope ratios of soil-CO_2 effluxes ($\delta^{13}C_{resp}$ and $\delta^{18}O_{resp}$) are commonly estimated based on a simple mixing model of soil-respired CO_2 and atmospheric CO_2 (Keeling, 1958). For this purpose, several methods have been developed to sample air with different fractions of soil-derived CO_2 using various types of chambers (Miller et al., 1999; Ekblad & Högberg, 2001; Torn et al., 2003; Bertolini et al., 2005) or 'mini towers' (Mortazavi et al., 2004; Kayler et al., 2010). So far, the most frequently applied method has been the use of static closed soil chambers from which air samples are collected at selected time intervals and analysed for $\delta^{13}C$ and $\delta^{18}O$ with an isotope ratio mass spectrometer (IRMS) (e.g. Ohlsson et al., 2005). The value for $\delta^{13}C_{resp}$ and $\delta^{18}O_{resp}$ is then given by the intercept of a simple linear regression through the plot of the stable isotope ratios and the reciprocal values of the CO_2 concentrations, the so-called Keeling plot.

An increasing number of studies, however, are challenging the strict linearity of Keeling plots derived from closed soil chambers and suggest errors in the estimates of $\delta^{13}C_{resp}$ and $\delta^{18}O_{resp}$ of up to several per mil. Mortazavi et al. (2004), for instance, observed that the $\delta^{18}O$ of chamber CO_2 was greatly affected by the $\delta^{18}O$ of the surface soil water as a result of an isotopic equilibration. Furthermore, the non-steady-state conditions in the chamber due to rising CO_2 concentrations may retard the diffusion of $^{12}CO_2$ more strongly than that of the heavier $^{13}CO_2$, leading to a slight concave-up curvature of the Keeling plots (Risk & Kellman, 2008; Ohlsson, 2009). In various theoretical simulations, Nickerson & Risk (2009b) compared different types of closed chamber methodologies and found that each may estimate biased values for $\delta^{13}C_{resp}$. Nevertheless, such a bias has not been reported in closed chamber studies before (e.g. Flanagan et al., 1999; Ngao, 2005; Ohlsson et al., 2005; Betson et al., 2007), possibly because of measurement uncertainties or due to the limited number of gas samples used.

In the last decade, emerging spectroscopic techniques have made it possible to measure $^{12}CO_2$, $^{13}CO_2$ and $^{12}C^{16}O^{18}O$ concentrations continuously with a high temporal resolution. Most recently, laser spectroscopy has been applied in the field to estimate $\delta^{13}C_{resp}$ (Bahn et al., 2009; Marron et al., 2009; Plain et al., 2009) and $\delta^{18}O_{resp}$ (Powers et al., 2010). All of these studies employed a mid-IR tunable diode laser-based spectrometer (TDLS) to make real-time measurements of the concentration and isotopic composition of CO_2 at the inlet and outlet of an open soil-chamber system. In contrast to closed chambers, the open chamber technique ensures steady-state conditions between chamber headspace and soil for several

minutes, which minimises one-dimensional chamber-to-soil feedbacks (Hutchinson & Livingston, 2002; Pumpanen et al., 2004; Midwood et al., 2008). Moreover, it allows the CO_2 effluxes and their isotopic signatures to be measured more frequently than by the closed chamber approach (Bahn et al., 2009; Powers et al., 2010). This, in turn, can help to reduce the uncertainty of the estimated values for $\delta^{13}C_{resp}$ and $\delta^{18}O_{resp}$. On the other hand, open chambers have potential flaws, which must be considered properly in the experimental design: (1) wind-induced pressure pumping in the chamber can create serious problems (Kutsch et al., 2001); (2) flow rates through the chamber must be measured very accurately; (3) estimates of $\delta^{13}C_{resp}$ and $\delta^{18}O_{resp}$ are based on one single mixing ratio of soil-derived and ambient CO_2 (chamber outlet) and are thus sensitive to small measurement errors, particularly when the difference in the CO_2 concentrations between chamber inlet and outlet is small (Midwood et al., 2008; Powers et al., 2010); (4) in- and outlet are typically measured sequentially requiring constant conditions within the measurement interval. Closed soil-chamber systems do not have the same challenges. Consequently, they require a less complex chamber design and are, in particular, easier to handle in the field.

In our study, we coupled a dynamic closed chamber system with a quantum cascade laser-based spectrometer (QCLS). This new class of laser offers some advantages over mid-IR TDL, especially for long-term field applications: (1) QC lasers operate at non-cryogenic temperatures (240–330 K), i.e. within the range of thermoelectric coolers (TEC), (2) they are not sensitive to thermal cycling and (3) they have an excellent single-mode behaviour and much higher power output than the lead salt diode laser, which is usually used in the mid-IR. The QCLS continuously measures the CO_2 isotopic composition in the chamber headspace during the CO_2 accumulation with a temporal resolution of one second. This new method, therefore, provides deeper insights into the chamber dynamics of $^{12}CO_2$, $^{13}CO_2$ and $^{12}C^{16}O^{18}O$ than ordinary chamber studies using IRMS. Furthermore, it allows $\delta^{13}C_{resp}$ to be determined not only from high resolution Keeling plots but also from the flux ratio of $^{12}CO_2$ and $^{13}CO_2$. The flux-ratio method has already been used to estimate $\delta^{13}C_{resp}$ from isotopic gradients over agricultural field sites (Griffis et al., 2004; Drewitt et al., 2009), but, to our knowledge, it has never been applied in soil chamber studies.

We evaluated our measurement system in a litter-manipulation experiment, where it was essential to determine $\delta^{13}C_{resp}$ with a high precision in order to calculate the litter fraction of soil respiration. The main objectives of this study were to: (1) apply for the first time a QCLS in a closed soil-chamber system, (2) analyse the dynamics of $\delta^{13}C$ and $\delta^{18}O$ in the chamber

headspace on a time scale of seconds, (3) assess the linearity of chamber-derived Keeling plots, and (4) examine different approaches to estimate $\delta^{13}C_{resp}$ and $\delta^{18}O_{resp}$.

2. Materials and methods

2.1 Experimental setup

The field experiment was performed on the Lägeren research site (CH-Lae in CarboEurope IP) situated at 47°28'40.8'' N, 8°21'55.2'' E in a productive beech forest (685 m a.s.l.) in the north-eastern part of the Swiss Jura. At this site, the bedrock is marl overlaid with limestone debris. The soils are calcareous Cambisols. The soil properties of the top soil (0–10 cm) are given in Table 1. During the measurements at the end of April 2008, the mean soil temperature at the depth of 10 cm was 10.4°C (ibuttons, Maxim Integrated Products DS1922L). The air temperature ranged from 7 to 17°C and the volumetric soil water content, measured at a meteorological station next to the experimental site (~ 50 m), was at around 35 vol-%.

Table 1. *Properties of the top soil 0–10 cm. Soil cores (5 cm diameter) were taken from the centre of five soil collars.*

pH ($CaCl_2$)	Particle-size distribution (%)			Fine earth bulk density (g cm^{-3})	C_{org} (%)	C/N	C_{org} pool (kg m^{-2})	$\delta^{13}C_{org}$ (‰)
	250-2000 µm	2-250 µm	< 2 µm					
7.5	25	21	54	0.91	3.9	12.0	3.6	–27.2

We employed our closed soil-chamber system in an ongoing litter experiment. Four different litter treatments were established in November 2007: (1) soil without any litter layer ('bare soil'), (2) soil + unlabelled beech leaves ($\delta^{13}C$: –31.1 ±0.2‰, 0.75 kg m^{-2}), (3) soil + ^{13}C-depleted beech leaves (–40.8 ±0.2‰, 0.75 kg m^{-2}), (4) soil + ^{13}C-depleted beech twigs (–38.4 ±0.1‰, 2 kg m^{-2}). The number of replicates was ten for the ^{13}C-labelled treatments and five for the unlabelled treatments. The replicates were arranged in five blocks within a radius of 12 m. Each litter plot was framed by acrylic glass (10 × 50 × 50 cm), separated from the surrounding soil by a trench to circumvent root respiration and had a PVC collar (20 cm in diameter, 10 cm high) for the CO_2-efflux measurements. The soil collars were inserted into the soil to a depth of 3 cm.

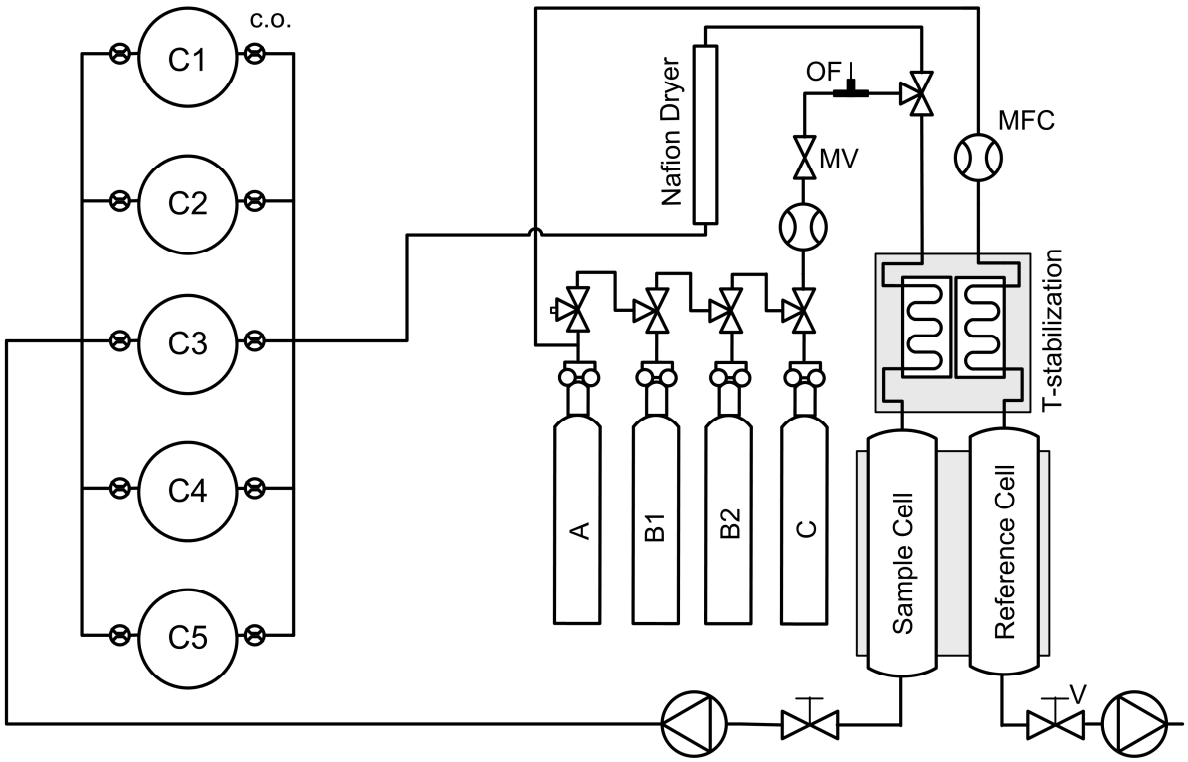

Figure 1. *Configuration of the closed chamber system and pathway of the chamber air for the online measurements with a QCL-based spectrometer. Notation used: C_i – soil chambers; A, B1, B2, C – reference gases; MFC – mass flow controller; MV – magnetic valve; V – manual precision valve; OF – overflow; c.o. – critical orifice.*

In contrast to most chamber studies, we did not measure the soil-CO_2 efflux in each soil collar separately, but pooled together the effluxes of five replicates of the same treatment using parallel-connected closed soil chambers (Fig. 1), which were placed simultaneously on the soil collars. This approach reduced the air flow through single soil chambers from one L min^{-1}, as required for the spectrometer, to 0.2 L min^{-1} and, consequently, minimized potential pressure variations in the chamber (Fang & Moncrieff, 1996). Moreover, it allowed the integration over a larger soil area within a single chamber measurement.

The dynamic closed soil chambers (20 cm in diameter, 6 cm high) had a similar design to those described by Fang & Moncrieff (1996). On the top of the chambers was a balance tube, 0.8 m in length and 0.5 cm in diameter, to equilibrate small pressure differences between chambers and atmosphere. Additionally, critical flow orifices (Lenox Laser, USA) inserted at the inlet and the outlet of the chambers ensured that the same amount of air entered into the

chambers as was drawn out. However, we installed no recirculation system in the chambers since it has been shown that the turbulence generated by fans can affect the CO_2 effluxes (Hanson et al., 1993; Pumpanen et al., 2004).

The sample gas from the five chambers was merged through Teflon tubing (I.D. 4 mm) and then drawn into the QCLS with an oil-free, heated and PTFE-coated diaphragm vacuum pump (N036ST.26E, KNF, Germany). The spectrometer was kept in an air-conditioned cottage about 80 m from the experimental site. The gas flow from the chamber system, comprising a total air volume of 22.5 L, was maintained at one L min^{-1}, resulting in a lag time of 50 s between the chambers and the spectrometer. Although pressure variations in the chamber headspace were not monitored, they should be well below one Pa, since the differences in air flow between chamber inlet and outlet, measured with a digital flow meter (GSM, Vögtlin Instruments, Switzerland), were less than 0.2%.

We analysed the soil-CO_2 efflux and its isotopic composition from April 18–28, 2008, by repeatedly closing the chamber system for 20 min time intervals. The different 'soil + litter' treatments were measured in a fixed sequence: (1) Bare soil; (2) soil + unlabelled leaves; (3) soil + twigs; (4) soil + twigs (2^{nd} five replicates); (5) soil + leaves; (6) soil + leaves (2^{nd} five replicates). After each measurement, the five soil chambers were moved to the soil collars of the next treatment. Our system allowed two measurement cycles per hour and, hence, repeated measurements of each treatment with an interval of three hours. However, at the beginning of the field campaign, heavy rainfall created serious problems for the sampling system by clogging the critical orifices. These data were therefore discarded and we focused our attention on the last two days, during which 90 measurement cycles were performed.

In addition to the regular 20-min sampling, we performed the following measurements at the end of the campaign: (1) The $\delta^{13}C_{resp}$ of the labelled litter without soil, referred to as 'litter only', was determined using collars with a lattice on the bottom. Here, the collars with decomposing litter were placed in a container with an impermeable surface that was closed with one soil chamber for 20 min. (2) One 45-min measurement was performed in both the 'soil + twigs' treatment and the 'twigs only' collars in order to examine the effects of a long-term CO_2 accumulation on the $\delta^{13}C$ and $\delta^{18}O$ dynamics in the chamber system.

2.2 Instrumentation

The CO_2 isotopic composition was measured with a QCLS, described in detail by Tuzson et al. (2008b). Briefly, the instrument employs a pulsed QCL (Alpes Lasers, Switzerland) as IR-light source operating at 15°C. The emitted laser beam is collimated by a reflecting objective

and then split into two parts of equal intensity. Each beam is coupled in a modified, small volume multi-pass cell (AMAC-36, Aerodyne Research Inc., USA, 7.6 m path length) and finally focused onto a pair of thermoelectrically cooled miniature IR-detectors (Vigo System, PL). The dual-multipass cell arrangement allows the sample and reference gases to be measured simultaneously and, hence, improves the accuracy of the isotope ratio determination by continually referencing the stable isotope ratios of the samples to the reference gas. The laser is tuned over the absorption features of the three main CO_2 isotopologues located near 2311 cm^{-1}. This is the same spectral window as first reported by Tuzson et al. (2008a). Laser scanning, signal processing and quantitative spectral fitting are fully automated through a dedicated PC software (TDLWintel, Aerodyne Research Inc., USA).

Absolute CO_2 concentrations and isotope ratio values were obtained after post-processing the raw data with Igor Pro 6 (WaveMetrics, Inc., USA), using the values from the calibration sequence (see below) and applying the approach described by Tuzson et al. (2008b). Given the linear behaviour of the analyzer, the entire calibration could be performed by measurements of three different reference gases. Two were needed to determine the dependency of measured isotope ratios on the CO_2 concentration, and a third to define the calibration function that links the raw spectroscopic isotope ratio values to the δ-scale. The

Table 2. *List of calibration gases used in this study. These gases were specially prepared for soil respiration measurements, i.e. considering the large range in the CO_2 mixing ratio and $\delta^{13}C$ values, respectively. For this purpose, two pure (100%) CO_2 gases from distinctively different sources (marine carbonate and methane burning) were diluted by high purity (5.0) synthetic air (O_2: 20.5%, N_2: 79.5%, H_2O < 5 μmol mol^{-1}) and then analysed by IRMS.*

Calibration gas	CO_2 (µmol mol^{-1})	$\delta^{13}C$ (‰)	$\delta^{18}O$ (‰)
Tank A[1)]	409.1 (0.1)	-10.30 (0.1)	-8.4 (0.2)
Tank B1	937.7 (0.2)	-3.75 (0.05)	-15.8 (0.1)
Tank B2	441.1 (0.1)	-3.75 (0.05)	-15.8 (0.1)
Tank C	446.5 (0.1)	-29.21 (0.05)	-24.5 (0.1)

[1)] Measured in the field by QCLS. This cylinder contained pressurized air which continuously flew through the reference cell (see text). It was used for referencing the sample and calibration gases as well as for monitoring instrumental drifts.

working standards used in this study were previously characterized in the laboratory (see Table 2). Their δ-values were determined by high precision IRMS analysis and expressed in relation to the VPDB-CO_2 scale (Werner et al., 2001), while the CO_2 concentration was linked to the World Meteorological Organization (WMO) scale.

Before entering the absorption cell, the sample gas was dried by a Nafion drier (PD-100T, PermaPure, USA), to avoid density effects and spectroscopic collisional broadening effects on the mixing ratio due to water vapour and filtered with a 7 μm sintered metal filter (Swagelok, USA). Afterwards, the gas was passed across a temperature stabilization module, which consists of copper tubes pressed into a channelled aluminium plate. This system is pre-heated and actively temperature controlled so that the gas temperature on exit closely matches the multi-pass absorption cell temperature in the optical module. Since both the sample and calibration gas flows parallel through this unit, the temperature difference between these gases is also minimized. This significantly reduces the errors in the isotope ratio measurements (Tuzson et al, 2008b). The flow rates and pressure (80 hPa) in both cells were controlled by combining high precision thermal mass-flow controllers (Vögtlin Instruments, Switzerland) and metering bellows-sealed valves up- and downstream of the cell (Fig. 1). The instrument's sampling system periodically switched between calibration gases and air sample. The measurement cycle was as follows: (1) standard tank A; (2) standard tank B1; (3) standard tank C; (4) standard tank B2; (5) air sample. Within each 60-min cycle, the calibration gases were measured for 150 s each and the sample air was analysed for 50 min (Fig. 2). During the air-sampling interval, the chamber system was closed twice for 20 min. Between two measurement cycles, the chamber system was opened for three minutes and purged with ambient air. During the ten minutes automated calibration sequence, the chambers were open. This cycling was applied to the sample cell only, while a continuous flow (0.18 L min^{-1}) of reference gas (tank A) through the reference cell was maintained during the entire campaign.

The precision of the instrument was characterized at the site under field sampling conditions by the Allan variance technique (Werle et al., 1993). For this, ambient air was continuously measured over 24 hours and then the corresponding Allan variance plot was calculated for the calibrated isotope ratio time series. This resulted in a conservative value for $\delta^{13}C$ of 0.25‰ at 1 Hz and a variance minimum of 0.05‰ after 100 s averaging time. Similar results were obtained for the ^{18}O isotope ratio.

2.3 Data analysis

All statistical analyses were performed in R-language (v 2.8.1). Each measurement cycle resulted in $\delta^{13}C$- and $\delta^{18}O$-Keeling plots, comprising 1200 data points (Fig. 3 and 4). The $\delta^{13}C$ of the soil-respired CO_2 ($\delta^{13}C_{resp}$) was estimated from Keeling plots by simple linear regression, using generalized least square models (Model I regression, see Zobitz et al., 2006) from the R-library 'nlme'. The regression models considered the autocorrelation structures of the residuals, estimated with AR-5 processes.

In addition to the Keeling plot method, we estimated $\delta^{13}C_{resp}$ from the flux ratio of $^{13}CO_2$ and $^{12}CO_2$ as follows:

$$\delta^{13}C_{resp} = \left(\frac{F^{13}CO_2 / F^{12}CO_2}{R_{VPDB}} - 1 \right) \times 1000‰ \quad (1)$$

where $F^{13}CO_2$ and $F^{12}CO_2$ are the effluxes of both isotopologues and R_{VPDB} is the standard molar ratio of ^{13}C and ^{12}C. The flux rates were determined by fitting the increase in the CO_2 concentration with (1) simple linear regression and (2) with a second-order polynomial function. For the later the flux rates were calculated as the first derivative at the start of the measurement. To avoid potential artefacts derived from turbulences by the closing of the chambers, the first minute of each 20-min measurement was omitted for the determination of the flux rates.

None of the above mentioned methods, however, is suitable to determine $\delta^{18}O_{resp}$ because the CO_2 in the chamber headspace air is affected by both the respiration and the isotopic exchange between CO_2 and the surface soil water, leading to a curved Keeling plot (Tans, 1998; Mortazavi et al., 2004). Therefore, we applied the mathematical concept proposed by Tans (1998) to determine the $\delta^{18}O$ signature of the soil-respired CO_2. Briefly, we plotted the $\delta^{18}O$ values against the concentration ratio of 'background' CO_2 and chamber CO_2 (C_o/C), as shown in Figure 4, and fitted the data points with a quadratic curve:

$$\delta^{18}O = \delta_o + a_1(C_o/C - 1) + a_2(C_o/C - 1)^2 \quad (2)$$

where δ_o is the $\delta^{18}O$ value of the 'background' CO_2. Based on the known parameters a_1 and a_2, equations (3) and (4) were then numerically solved for δ_{eq} and A:

$$a_1 = -(\delta^{18}O_{resp} - \delta_o) - A(\delta_{eq} - \delta_o), \quad (3)$$

$$2a_2 = -A(\delta^{18}O_{resp} + \delta_{eq} - 2\delta_o) - A^2(\delta_{eq} - \delta_o), \quad (4)$$

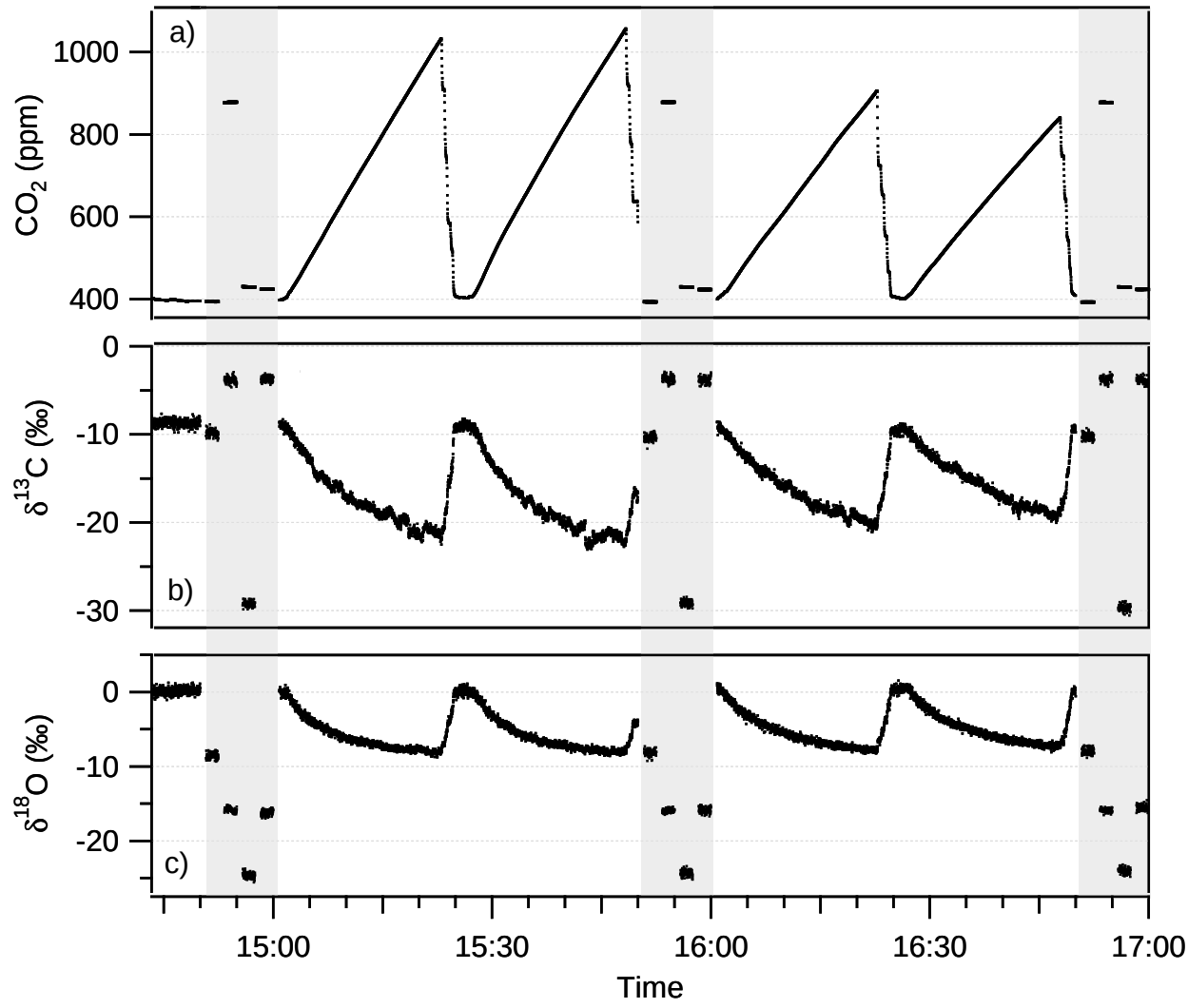

Figure 2. *Time series of the CO_2 concentration (a), the $\delta^{13}C$ of the CO_2 (b) and the $\delta^{18}O$ of the CO_2 (c) in the chamber system for four consecutive measurement cycles. Each measurement cycle lasted 20 minutes followed by a short purging with ambient air. After two cycles, a calibration sequence of 10 min was applied and four calibration gases were measured for 150 s each (grey band).*

The factor A is defined as the ratio between the total amount of chamber CO_2 that exchanged with the surface soil water and the amount of soil-respired CO_2. The $\delta^{18}O_{resp}$ can be expressed as the sum of the kinetic fractionation ($\varepsilon_D f$) assumed to be –8.7‰ when CO_2 diffuses out the soil (see Hesterberg & Siegenthaler, 1991; Tans, 1998), and the isotopic equilibrium value (δ_{eq}) of CO_2 with the moisture in the upper few cm of soil.

Variations in $\delta^{13}C_{resp}$ during single measurements were assessed with moving windows of 400 data points, which were moved in one-second steps. For each window, the intercept of the least square fit was estimated separately. The period for the window was selected based on the observation that the standard error of the intercepts only slightly decreased when the integration time for the least square fits was larger than 400 s.

The litter-derived fraction of the soil-CO_2 efflux was calculated by:

$$f_{litter} = (\delta^{13}C_{resp\_total} - \delta^{13}C_{resp\_soil}) / (\delta^{13}C_{litter} - \delta^{13}C_{soil}) \quad (5)$$

where $\delta^{13}C_{resp\_total}$ and $\delta^{13}C_{resp\_soil}$ are the ^{13}C signatures of the CO_2 effluxes from 'soil + litter' and from 'bare soil', respectively, whereas $\delta^{13}C_{litter}$ and $\delta^{13}C_{soil}$ are the ^{13}C signatures of the bulk litter and the soil organic matter in the top soil (0–10 cm), respectively. The effects of temperature and soil-CO_2 efflux on $\delta^{13}C_{resp}$ were assessed by analyses of covariance (ANCOVA), using the litter treatment as factor level and temperature and efflux as co-variables.

3. Results and Discussion

3.1 CO_2 dynamic in the chamber system

The CO_2 concentrations in the chamber system increased almost linearly from about 400 ppm to 700–1050 ppm during accumulation (Fig. 2). Simultaneously, the $\delta^{13}C$ and the $\delta^{18}O$ of the chamber CO_2 declined exponentially by 7 to 13‰ and by 5 to 9‰, respectively. The soil-CO_2 effluxes ranged from 1.5 to 3.2 μmol CO_2 m^{-2} s^{-1}, and, when averaged for the litter treatments, differed only about 5% from those measured with a mobile IRGA one week before at slightly lower soil temperatures (Table 3). Given this good agreement, we feel confident that our system measures soil respiration rates accurately.

High R^2 values (0.96–0.99) and standard errors of the intercept clearly below 0.1‰ indicated that simple linear regression fitted all $\delta^{13}C$-Keeling plots very well (e.g. Fig. 3). In contrast, the $\delta^{18}O$-Keeling plots were better described by quadratic curve fits (R^2 = 0.95–0.98) than by simple linear regression (R^2 = 0.88–0.96) as shown in Fig. 4.

While the concentrations and the ^{18}O ratios of CO_2 changed very regularly throughout the accumulation period (Fig 2a, Fig. 4), we observed pronounced oscillations in the $\delta^{13}C$ values (Fig. 3). Autocorrelation analyses revealed that the residuals of the Keeling plot regressions were significantly autocorrelated when a window larger than the initial 3 min was used. With rising CO_2 concentrations, the autocorrelation pattern intensified and, in the second part of the

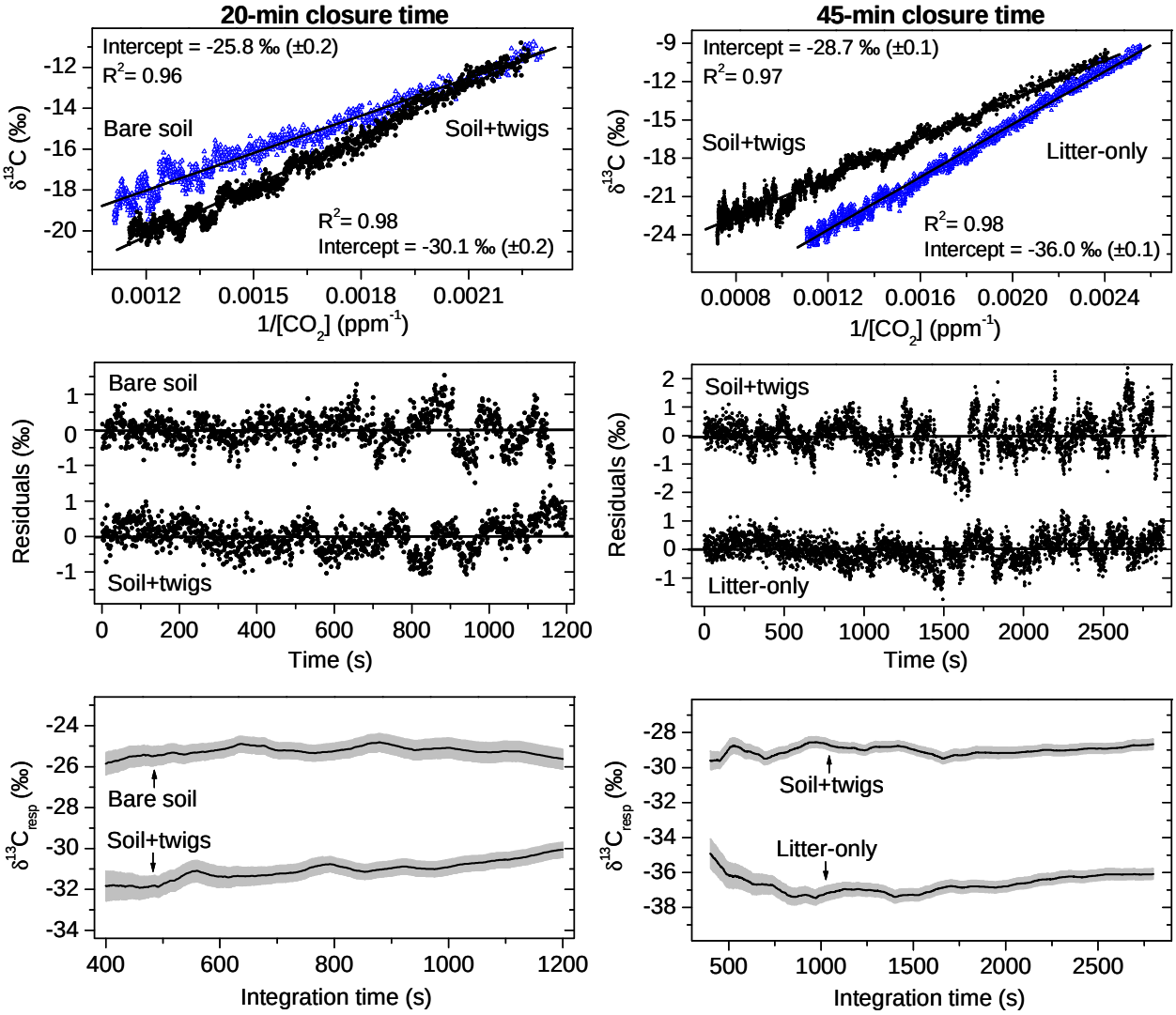

Figure 3. *$\delta^{13}C$-Keeling plots (upper figures) and corresponding residuals of the least square fits (middle figures) for the two 45-min measurements and for two typical 20-min measurements, one with a pronounced variability in $\delta^{13}C_{resp}$ ('soil + twigs') and the other with a small variability ('bare soil'). The figures below show changes in estimates of $\delta^{13}C_{resp}$ when an increasing number of data points is used for linear regression (from the first 400 s to the entire Keeling plot). The grey band represents the 95%-confidence interval of $\delta^{13}C_{resp}$. The linear regression models take into consideration the auto-correlation structure of the residuals.*

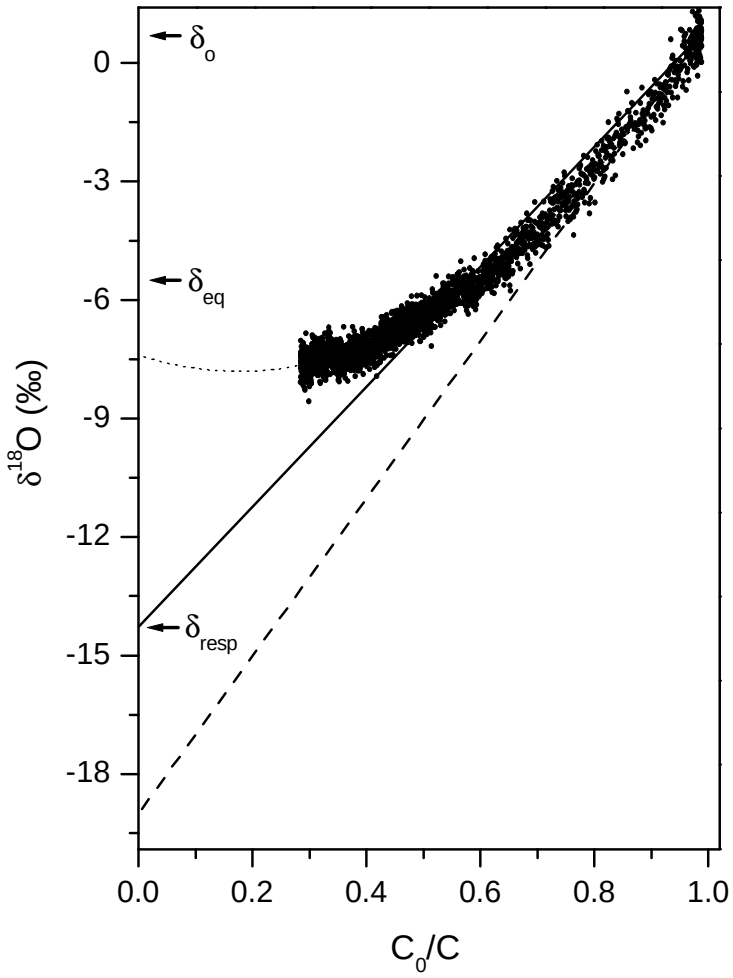

Figure 4. *Typical Keeling plot of $\delta^{18}O$ values. The isotope ratios are plotted against the concentration ratio of background air CO_2 and chamber CO_2. The ratio of the exchange flux to the respiratory flux (parameter A in Eq. 2 and 3) was 0.93 and the $\delta^{18}O$ of the soil water (δ_{eq}) was –5.7‰, leading to an isotopic ratio of soil-respired CO_2 ($\delta^{18}O_{resp}$) of –14.4‰. The dashed straight line is the tangential line calculated for the quadratic curve (dot line) at the ambient CO_2 concentration.*

20-min measurements, $\delta^{13}C$ values always started to oscillate. Toward the end of the 45-min measurement, the $\delta^{13}C$ values fluctuated by as much as ±1.5‰ (Fig. 3) despite a constant increase in the CO_2 concentration. The generalized least square models take into consideration the autocorrelation structure, thereby yielding the same intercepts but with about three times higher standard errors than ordinary linear regression.

The oscillations in the $\delta^{13}C$ could not be explained by an instrumental instability as we found no fluctuations in the gas temperature, the pressure, the laser intensity or in the $^{12}C^{16}O_2$ and $^{12}C^{16}O^{18}O$ signal. This finding is supported by the fact that the $\delta^{13}C$ values were no longer

Table 3. *Soil respiration (R_{total}), litter-derived CO_2 effluxes (R_{litter}) and litter fractions of R_{total} (f_{litter}) calculated from both QCLS measurements and ordinary chamber measurements using IRMS and a mobile IRGA (LI-COR 8100). The IRMS measurements were performed for each replicate separately one week before our experiment (11 am–16 pm) using the same soil chambers with a closure time of 20 min. The QCLS values are campaign averages (±SE). The IRMS values are means of 10 replicates (±SE).*

	R_{total} (µmol CO_2 m^{-2} s^{-1})	R_{litter} (µmol CO_2 m^{-2} s^{-1})	f_{litter} (%)	T-surface (℃)
Soil+leaves				
QCLS	1.81 (0.1)	0.25 (0.1)	14 (2)	11.0
IRMS	1.67 (0.1)	0.23 (0.1)	14 (2)	9.0
Soil+twigs				
QCLS	2.32 (0.1)	0.54 (0.1)	23 (2)	11.2
IRMS	2.37 (0.2)	0.68 (0.1)	29 (3)	9.3

autocorrelated immediately after the chamber system was opened and ambient air was measured. Moreover, no autocorrelations were observed in an additional laboratory experiment without a chamber system, where we varied the CO_2 concentrations of a standard gas from 250 ppm to 1100 ppm by dynamically diluting it with standard air.

The occurrence of oscillations in the 'litter-only' treatments measured on an impermeable surface without soil (Fig. 3) suggests that the fluctuations in the $\delta^{13}C$ were probably an artifact of the chamber system and were not caused by either short-term alterations in the CO_2 source or the advection of additional soil CO_2 with varying $\delta^{13}C$ values induced, for instance, by wind-driven pressure pumping (see Takle et al., 2004; Bain et al., 2005). Furthermore, both the missing oscillations in the $\delta^{18}O$ (Fig. 4) and the constant increase in the CO_2 concentrations (Fig. 2a) clearly exclude the possibility of a leak in the chamber system that would otherwise have led to an invasion of ambient air.

A potential mechanism for the oscillation in $\delta^{13}C$ could have been the stratification of CO_2 with different ^{13}C ratios in the chamber headspace due to insufficient mixing of the chamber air as no recirculation system was used. The oscillations in the $\delta^{13}C$ could then have resulted from intermittent advection of CO_2 from different strata to the chamber outlet.

3.2 Systematic error in $\delta^{13}C_{resp}$

Recent simulation studies suggest that the non-steady-state conditions in closed soil-chambers create slight non-linearities in the $\delta^{13}C$-Keeling plot, leading to systematic overestimations of the $\delta^{13}C$ of the soil-CO_2 efflux ($\delta^{13}C_{resp}$) (Risk & Kellman, 2008; Ohlsson, 2009). In ordinary Keeling plot studies, such a bias is difficult to recognize because only a limited number of gas samples is used. In our study, however, we were able to assess if the Keeling plots are strictly linear by performing 800 least square fits for each measurement cycle with an increasing number (from 400 to 1200) of data points (Fig. 3). The maximum spread of these 800 intercepts ranged from 1 to 2.5‰ and in most Keeling plots exceeded the 95% confidence interval of the intercept extrapolations, indicating that $\delta^{13}C_{resp}$ varied significantly during the CO_2 accumulation. Although the temporal pattern of these variations was not identical for all measurement cycles (see Fig. 3), there was a significant positive shift of 0.7‰ for $\delta^{13}C_{resp}$ when only the first 10 min were used for the linear regression instead of the entire 20 min ($p < 0.001$, Wilcoxon signed rank test).

Intercepts extrapolated from moving windows of 400 s revealed that on average, $\delta^{13}C_{resp}$ did not vary systematically for the first 13 min, which corresponds with a CO_2 build up of 300 ppm (Fig. 5). Toward the end of the measurements, however, $\delta^{13}C_{resp}$ significantly increased: On average, least square fits estimated 1.9‰ higher $\delta^{13}C_{resp}$ when the last 400 s out of 1200 s were used instead of the first 400 s ($p < 0.001$; Wilcoxon signed rank test). The shift in $\delta^{13}C_{resp}$ was very similar in all treatments (Fig. 5), suggesting that the estimation of litter-derived CO_2 was only slightly affected by the bias in $\delta^{13}C_{resp}$.

One reason for the non-linearity in the ^{13}C-Keeling plots could have been the dissolution of chamber CO_2 in soil water of the first centimetres and the accompanying ^{13}C fractionation, since the soil water had pH values of about 7.5 throughout the soil profile. Assuming that the chamber CO_2 invaded to a depth of 5 cm into the soil which had a volumetric water content of 35%, we found that the dissolution of accumulated CO_2 was at most 8% of the chamber CO_2. At a CO_2 increase of 450 ppm, the resulting ^{13}C enrichment of the chamber CO_2 would have been 0.4‰, based on the fractionation value of 9.6‰ for the equilibrium fractionation at the temperature of 10.4°C (Zhang et al., 1995). Such ^{13}C enrichment would have been enough to cause the observed non-linearity. However, it is unlikely that 8% of the soil-respired CO_2 was dissolved in soil water since water was present in macro- and micropores and not as a free body of water. Therefore, we believe that the non-linear pattern in the Keeling plots can rather be attributed to the so-called ‘diffusive kinetic fractionation’ as recently described by Nickerson & Risk (2009a). They show that chamber-to-soil feedbacks reduce the flux rate of

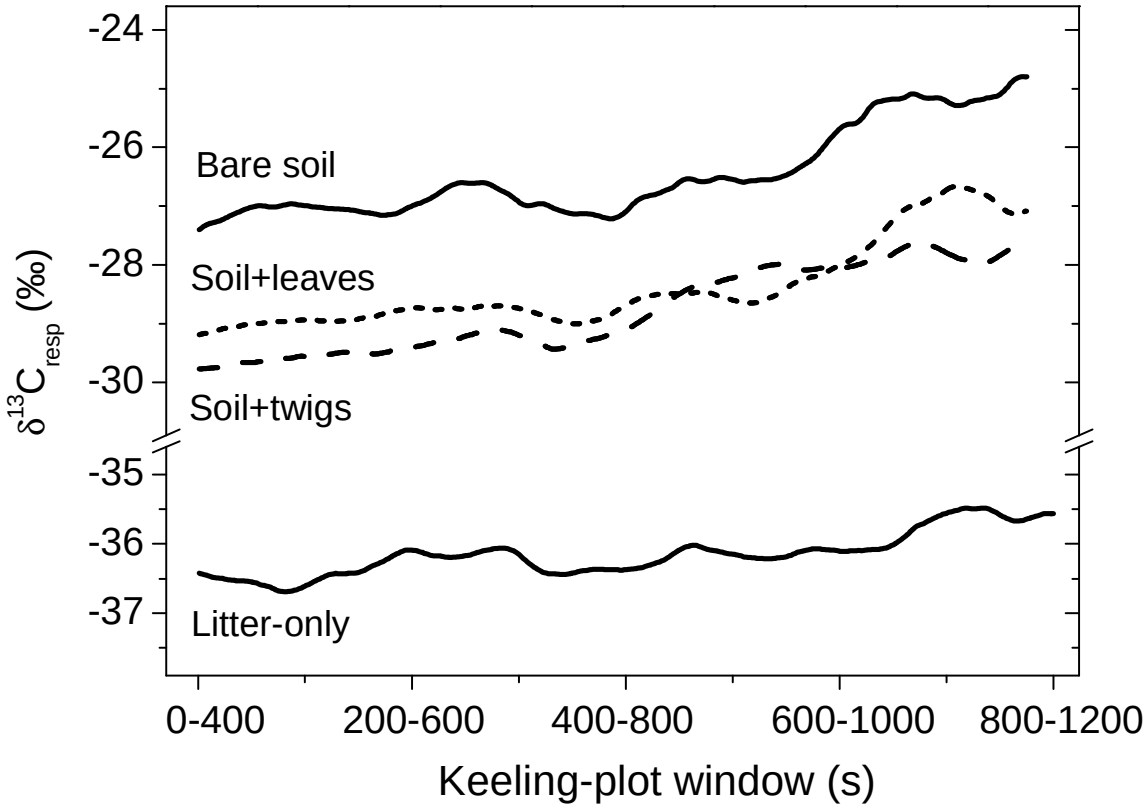

Figure 5. *Temporal course of $\delta^{13}C_{resp}$ for 20 min of CO_2 accumulation, estimated with 'moving windows' of 400 data points. The curves represent mean values for the different treatments (bare soil: n = 12; soil + leaves: n = 30; soil + twigs: n = 31; litter-only: n = 6). Please not the break in the y-axis.*

the faster diffusing $^{12}CO_2$ in comparison to that of $^{13}CO_2$, leading to significant concave-up curvature of the Keeling plots. The curvature was most pronounced when the model included the lateral diffusion of CO_2 around the chamber. As a consequence, simple linear regression overestimated $\delta^{13}C_{resp}$ by up to 2‰ for chamber-closures of 20 min. Their 'worst case' scenario, however, was based on considerably lower CO_2-production rates and higher diffusivity rates than in our soils. Surprisingly, we found no relationship between the degree of the bias in $\delta^{13}C_{resp}$ and the CO_2 effluxes ($p = 0.88$), which suggests that the closure time rather than the amount of accumulated CO_2 affected $\delta^{13}C_{resp}$. This assumption, however, was not supported by the 45-min measurement, where the bias in $\delta^{13}C_{resp}$ was not more pronounced than in the 20-min measurements (Fig. 3).

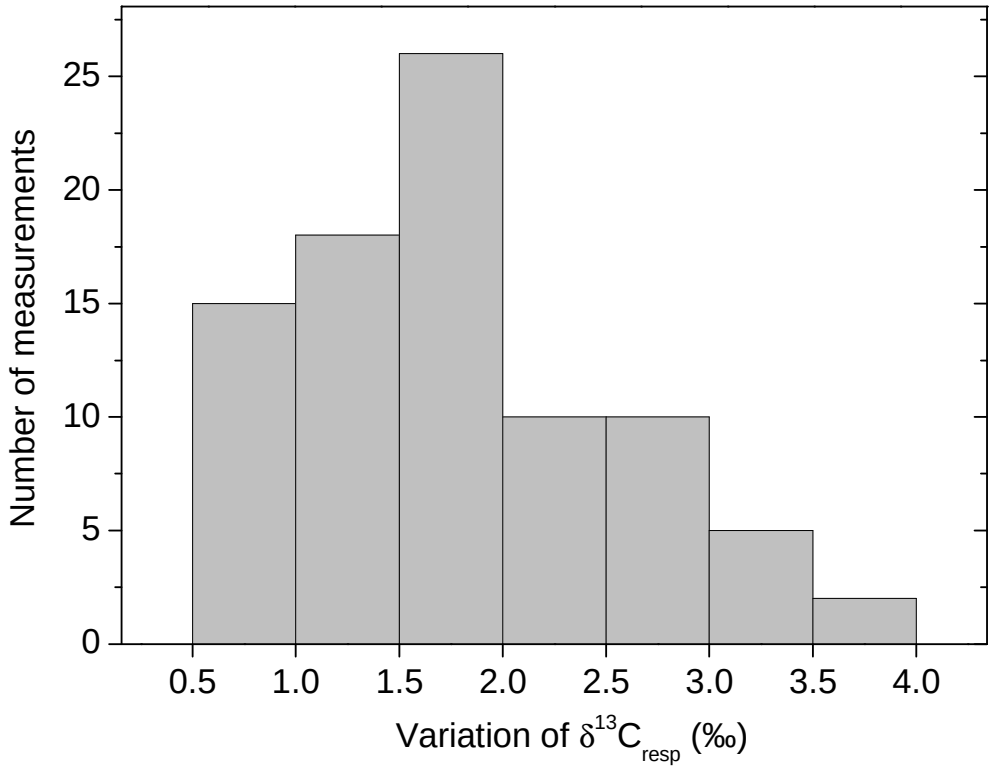

Figure 6. *Variation of $\delta^{13}C_{resp}$ for the first 10 min of CO_2 accumulation. The variation is the difference between the highest and lowest value of $\delta^{13}C_{resp}$, estimated from 200 Keeling plot using 'moving windows' of 400 s.*

In the litter-only measurements, we expected much smaller chamber artifacts since the chamber comprising the litter layer of only 3 cm were delimited from the surrounding soil and thus, diffusive kinetic fractionation was supposed to be clearly less important than in the normal soil chambers. Indeed, these Keeling plots differed less from strict linearity than those of the 'soil + litter' treatments (Fig. 5), even though a slight increase of 0.8‰ in $\delta^{13}C_{resp}$ was also observed towards the end of the measurements. This shift could indicate that even in the shallow litter layer a slight diffusive fractionation occurred.

3.3 Improving the estimates of $\delta^{13}C_{resp}$

The finding that $\delta^{13}C_{resp}$ was significantly biased only in the second part of the chamber measurements suggests that the correct value for $\delta^{13}C_{resp}$ could be determined by using only the first 600 data points. These shortened Keeling plots had both R^2 values and standard errors of the intercept that were equally good than those of the entire Keeling plots. The 'moving

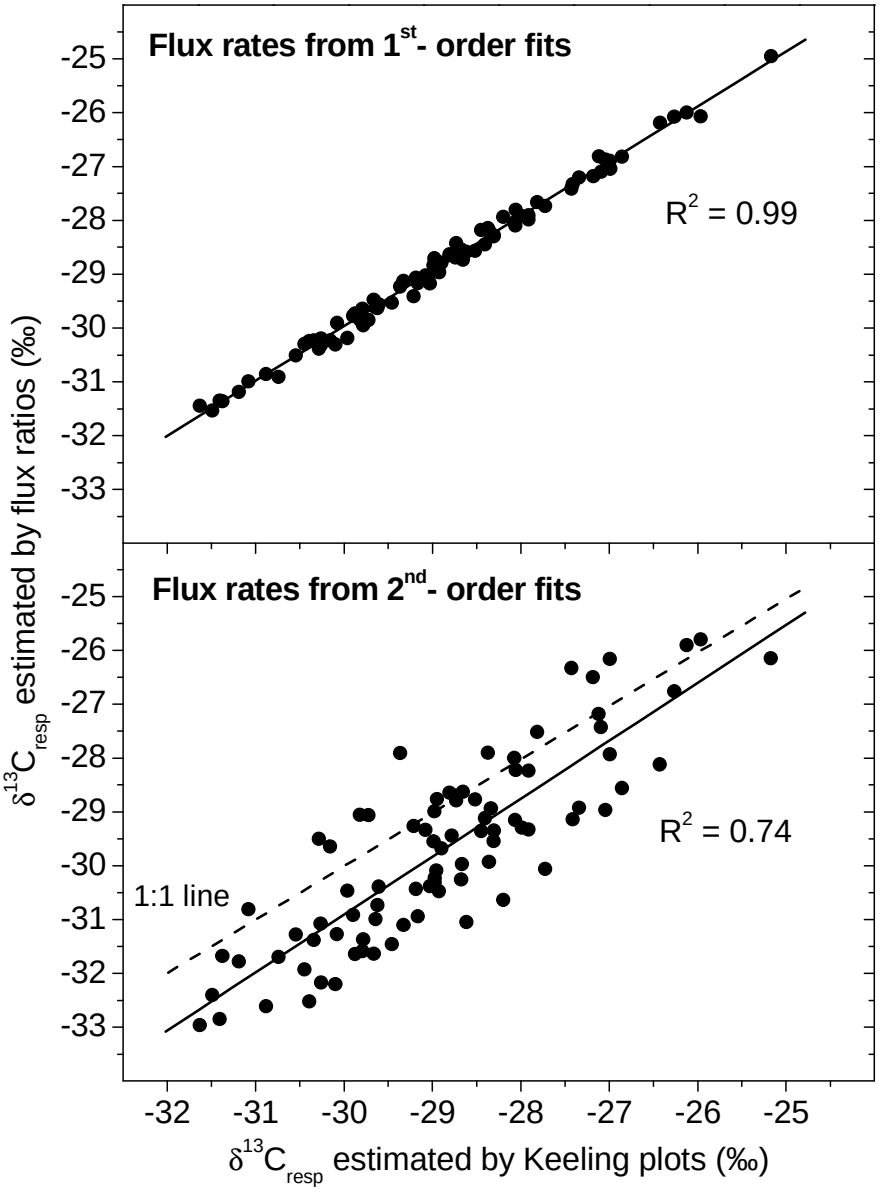

Figure 7. *Values for $\delta^{13}C_{resp}$, estimated by the flux-ratio method, plotted against the intercept of Keeling plots of the first 10 minutes of each measurement (n = 90). The flux rates were derived from both first and second order fits of the increase in the $^{13}CO_2$ and $^{12}CO_2$ concentrations between one and 10 min after the chamber system was closed.*

windows' of 400 s revealed, however, that the standard errors are limited in how well they indicate the uncertainty of the estimated $\delta^{13}C_{resp}$: While the errors were always below 0.3‰, the 200 values for $\delta^{13}C_{resp}$ obtained from the 'moving windows' showed a spread within single measurements of 1.8‰, on average, and in a few cases of up to 3.5‰ (Fig. 6). These non-systematic variations mostly exceeded the 95%-confidence interval.

In addition to the Keeling plot approach, we determined $\delta^{13}C_{resp}$ directly from the flux ratio of $^{13}CO_2$ and $^{12}CO_2$ using the concentration increase between the first and the tenth minute after the closure of the chamber system (Eq. 1). Here, the estimates for $\delta^{13}C_{resp}$ clearly depended on how the fluxes were calculated: (1) When the flux rates were derived from the slope of simple linear regressions, the flux-ratio method yielded values for $\delta^{13}C_{resp}$ very close to the Keeling plot intercepts (±0.05‰; $R^2 = 0.99$; Fig. 7). Hence, the application of the flux-ratio method in 'moving windows' of 400 s indicated the same shift in $\delta^{13}C_{resp}$ during the CO_2 accumulation as the Keeling approach (data not shown). Moreover, the negligible difference between the two approaches underlines that the extrapolation of the intercept contributes only little to the uncertainty of $\delta^{13}C_{resp}$ in these high resolution Keeling plots. (2) In contrast, when the flux rates were calculated from the first derivative of the quadratic curve fits of the increases in $^{13}CO_2$ and $^{12}CO_2$, then the flux-ratio values distinctly differed from the Keeling plot intercepts ($R^2 = 0.74$; Fig. 7). The absolute difference of about 1‰ can partly be attributed to the high sensitivity of the first derivatives to small uncertainties in the curve fit even when the R^2 values of the fit were above 0.99. Error propagation resulted in standard errors for $\delta^{13}C_{resp}$ which were on average 1.2‰ and, in a few cases, up to 2.5‰. Our results also show that the flux-ratio values were systematically lower than the Keeling plot intercepts (–0.8‰, $p < 0.001$, Fig. 7), which may indicate that there was a slight shift in $\delta^{13}C_{resp}$ even during the first minutes of the chamber measurements. This initial shift was probably not significant in the 'moving windows' of the linear approaches as they average across several minutes of CO_2 accumulation. Thus, the quadratic fit of the increase in $^{13}CO_2$ and $^{12}CO_2$ appears to be a promising approach to estimate $\delta^{13}C_{resp}$ accurately but only when an adequate number of replicates is used.

For both the Keeling and the flux-ratio approach, we observed maximum differences in $\delta^{13}C_{resp}$ of 3–5.5‰ between single measurements of the same treatment over two days (Fig. 8). The distinct variability in $\delta^{13}C_{resp}$ could not be explained by either plant-driven processes, as root respiration was excluded by trenching, or the daily cycle of the surface temperatures ($p = 0.79$). Therefore, the variability might have been driven by physical factors leading to advection and diffusion of ^{13}C enriched soil gas (Maseyk et al., 2009; Nickerson & Risk, 2009c; Kayler et al., 2010). Physical factors could have been: (1) pressure pumping of varying intensity due to an observed decrease in atmospheric pressure (–15 hPa) and/or wind events (Takle et al., 2004), (2) varying CO_2 effluxes (±20%), (3) changing CO_2 concentrations (395–435 ppm) and (4) changing ^{13}C ratios (–9.0 to –11.5‰) of ambient CO_2 at the soil surface. We found, however, no obvious relationships between these variations and

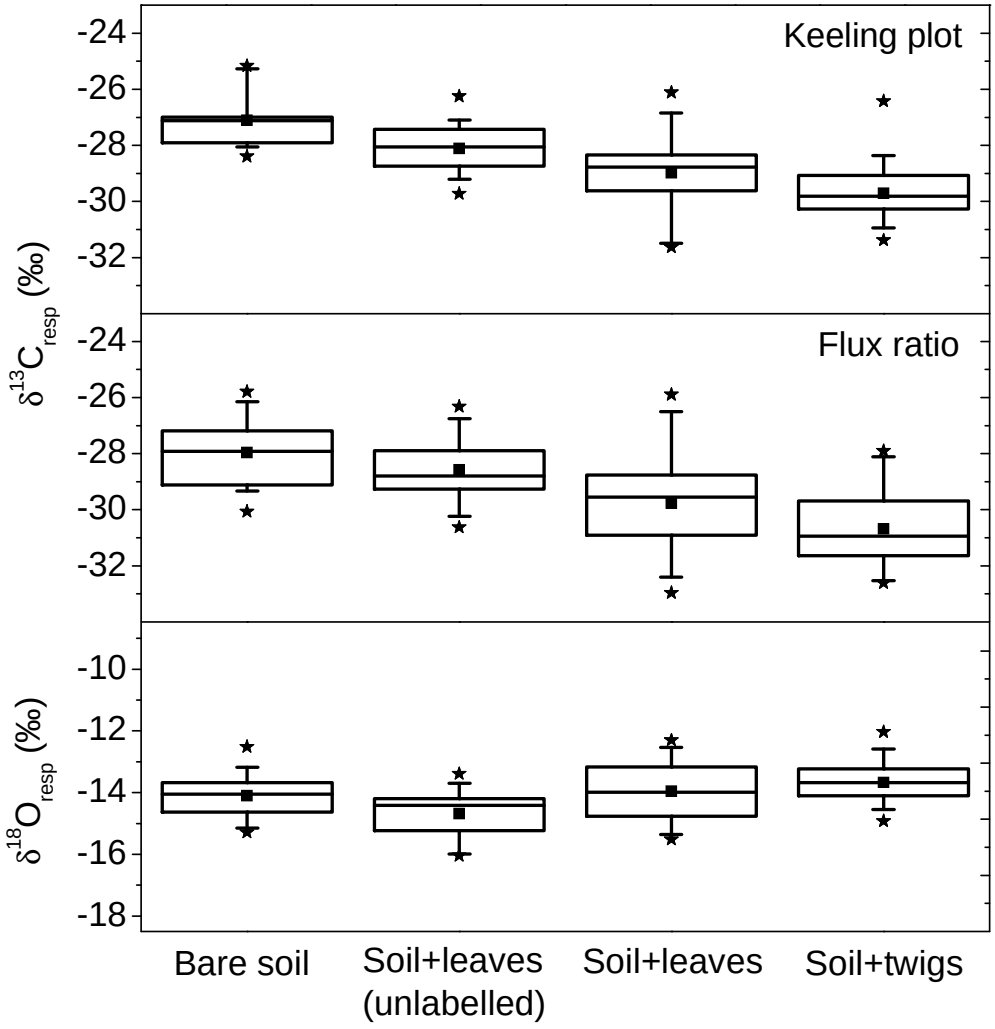

Figure 8. *Variability of $\delta^{13}C_{resp}$ (upper figures) and $\delta^{18}O_{resp}$ (lower figure) over two measurement days. $\delta^{13}C_{resp}$ was estimated from Keeling plots of the first 10 min and from the ratio of $^{13}CO_2$ and $^{12}CO_2$ effluxes calculated with quadratic curve fits of the CO_2 increase. $\delta^{18}O_{resp}$ was calculated from the curve fit of the Keeling plot (Eq. 2). The boxes show the median, the quartiles and the 2.5%- and 97.5%- quantiles. The squares are the mean values and the stars are the maximum values.*

those in $\delta^{13}C_{resp}$ derived from both Keeling plots and flux ratios and therefore we cannot exclude methodical uncertainties exceeding ±1‰. Such uncertainties must have resulted from the soil-chamber system as we observed no irregularities in the performance of the spectrometer.

The precision of our measurement system (±1‰) seems to be lower than that of open chamber systems (precision < 0.7‰) coupled to online stable isotope analyzers (TDLS or continuous flow IRMS) as recently tested in the laboratory (Midwood et al., 2008; Powers et

al., 2010). To our knowledge, however, the superior precision and accuracy of open chamber systems has not yet been confirmed by field measurements, although they have recently been applied to estimate diurnal cycles of $\delta^{13}C_{resp}$ using TDLS (Bahn et al., 2009; Maroon et al., 2009; Plain et al., 2009; Wingate et al., 2010). One potential problem is the lateral diffusion of CO_2 around the open chamber, which leads to ^{13}C enrichments of CO_2 effluxes, as it may happen in closed chambers (Nickerson & Risk, 2009a; Ohlsson, 2009). This chamber artifact could particularly be pronounced when the gradient in CO_2 concentrations (up to a few 100 ppm) between chamber and atmosphere persists over a longer period. In contrast to high resolution Keeling plots, such systematic errors are difficult to recognize in open systems because there is a constant difference in CO_2 mixing ratios between the in- and outlet of the chamber.

Despite the uncertainties of single measurements, we argue that our measurement system yields very accurate values for $\delta^{13}C_{resp}$ when several replicates are used because: (1) the mean ^{13}C signature of the CO_2 released from the 'bare soil' corresponded to those of the SOM in the mineral soil 0–10 cm (–27.1 ±0.3‰ vs. –27.2 ±0.2‰), which is reasonable from a mass balance point of view. In contrast, values for $\delta^{13}C_{resp}$ measured in other soil-respiration studies have often been less negative than the CO_2 source (Bowling et al., 2008). (2) In the 'soil+litter' treatments, the litter layer was clearly reflected in $\delta^{13}C_{resp}$ (Fig. 8). The mean values of $\delta^{13}C_{resp}$ were 0.9‰ and 1.6‰ smaller when ^{13}C depleted leaves ($\delta^{13}C_{litter}$ = –40.8‰, 750 g m^{-2}) and twigs ($\delta^{13}C_{litter}$ = –38.4‰, 2 kg m^{-2}) were added to soils than when unlabelled leaves were added ($\delta^{13}C_{litter}$ = –31.1‰, 750 g m^{-2}). The litter fractions of soil respiration, as well as the resulting litter-derived CO_2 fluxes, were in accordance with values found with ordinary IRGA and IRMS measurements one week before our experiment (Table 3).

3.4 Determination of $\delta^{18}O_{resp}$

In all 90 measurement cycles, the $\delta^{18}O$ of the soil-CO_2 efflux decreased immediately after the chamber system was closed, leading to a distinctly curved Keeling plot (Fig. 4). This ^{18}O enrichment can be explained by the invasion of chamber CO_2 into the first few cm of soil, where the ^{18}O of CO_2 equilibrates partly with the soil water before it diffuses back to the headspace. This process has been discussed in other studies (Tans, 1998; Flanagan et al., 1999; Miller et al., 1999), and has also been observed in static closed chambers (Mortazavi et al., 2004).

As a consequence, both the Keeling plot intercepts and flux ratios are not suitable to estimate $\delta^{18}O_{resp}$ (Tans, 1998). Our high resolution Keeling plots, however, allowed us to

apply the mathematical formalism provided by Tans (1998), using the parameters from the quadratic curve fits (Eq. 2) in Eq. 3 and 4. The mean value for A in Eq. 3 and 4 was 0.95 (±0.03), indicating that the amount of chamber CO_2 that equilibrated with the soil water was, on average, almost the same as the amount of respired CO_2. The vigorous isotopic exchange resulted from the long residence time of the chamber air over the soil, and agrees with the results from both a closed chamber study (Mortazavi et al., 2004) and an open chamber study, where the flow rate through the chamber was greatly reduced (Miller et al., 1999). In addition, the equilibration of the chamber CO_2 with the surface soil water might have been accelerated by the high volumetric water content (35%) due to the heavy rainfalls three days before the measurements.

The values calculated for the ^{18}O signature of the soil water with which soil-respired CO_2 equilibrated during CO_2 diffusion (δ_{eq}) ranged from –3.5 to –7.5‰. Unfortunately, we have no $\delta^{18}O$ data for the soil water to verify these values. The average δ_{eq} of –5.4‰, however, is in good agreement with values measured in spring in grass-covered soil near Bern (Switzerland) (Hesterberg & Siegenthaler, 1991). The intercept of a tangential line at ambient CO_2 (Fig. 4), would have been off by, on average, –5.4‰ from the real $\delta^{18}O_{resp}$ (–14.1‰), estimated by adding the value for the kinetic fractionation ($\varepsilon_D f$) of –8.7‰ to δ_{eq}.

The values estimated for $\delta^{18}O_{resp}$ showed variability similar to those for $\delta^{13}C_{resp}$ (Fig. 8). We think that the variability in $\delta^{18}O_{resp}$ can be partly explained by our assumption of a constant value for $\varepsilon_D f$. This value might have varied over the two days of measurements either due to an intermittent advection of soil CO_2 instead of diffusion or because CO_2 produced in the upper layer diffused out of the soil without full equilibration with the soil and litter water (Lin et al., 1999; Miller et al., 1999).

4. Conclusions and recommendations

Our study shows that QCL-based spectrometers can be applied in closed soil-chamber systems to measure the stable isotope ratios of accumulating CO_2 every second with a precision of 0.25‰. The resulting $\delta^{13}C$- and $\delta^{18}O$-Keeling plots, however, must be analysed carefully, as the CO_2 in the chamber system showed the following behaviours in our experiment: (1) the $\delta^{13}C$ of the CO_2 oscillated, which was probably an artifact of the chamber system. (2) $\delta^{13}C_{resp}$ was often not constant during the flux measurements and systematically shifted after a certain chamber-closure time (on average 10 min) due to chamber-to-soil feedbacks. (3) The $\delta^{18}O$ of the CO_2 in the chamber headspace was significantly affected by isotopic exchange with the upper soil water, leading to a curved $\delta^{18}O$-Keeling plot. Therefore,

the classical methods to estimate $\delta^{13}C_{resp}$, which are based on the linearity of the Keeling plot, estimated $\delta^{18}O_{resp}$ values inaccurately.

We also showed that for the estimation of $\delta^{13}C_{resp}$, the flux-ratio method can be used alternatively to Keeling plots. The flux ratio method might account better for the ^{13}C enrichments of soil-CO_2 effluxes in the chamber, e.g. due to diffusive fractionation, than the Keeling approach, but only when the isotopic flux rates are derived from quadratic fits. These estimates are, however, less robust than Keeling plots and, thus, only suitable when adequate numbers of replicates are used.

For future studies employing a closed soil-chamber system coupled with a continuous stable CO_2-isotopes analyzer, we would recommend the following: (1) Keep the closure time as short as possible to minimise the disturbance of the soil-atmosphere system. In our case, the optimal closure time of the chambers would have been 10 instead of 20 min, corresponding to an increase of about 230 ppm CO_2. (2) Screen each Keeling plot using the 'moving window' approach and reject measurements, in which the variability of $\delta^{13}C_{resp}$ during CO_2 accumulation clearly exceeds the confidence interval of intercept extrapolations. (3) Apply the flux-ratio method to support the Keeling plot approach and to detect biases in $\delta^{13}C_{resp}$. (4) Fit the $\delta^{18}O$-Keeling plots with a quadratic curve (Eq. 2) and use the parameters of this fit in Eq. 3 and 4 to approximate $\delta^{18}O_{resp}$. (5) Daily variations in the $\delta^{13}C_{resp}$ of 3–5.5‰ suggest that for the partitioning of soil respiration using small ^{13}C-label signals, control and treatment plots need to be measured consecutively within short time, or even simultaneously, with several replicates.

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References

Bahn M., Schmitt M., Siegwolf R., Richter A., Brüggemann N. (2009) Does photosynthesis affect grassland soil-respired CO_2 and its carbon isotope composition on a diurnal timescale?. New Phytologist, 182, 451–460.

Bain W. G., Hutyra L., Patterson D. C., Bright A. V., Daube B. C., Munger J. W., Wofsy S. C. (2005) Wind-induced error in the measurement of soil respiration using closed dynamic chambers. Agricultural and Forest Meteorology, 131, 225–232, doi:10.1016/j.agrformet.2005.06.004.

Bertolini T., Inglima I., Rubino M., Marzaioli F., Lubritto C., Subke J. A., Peressotti A., Cotrufo F. (2005) Sampling soil-derived CO_2 for analysis of isotopic composition: a comparison of different techniques. Isotopes in Environmental and Health Studies, 42 (1), 57–65.

Betson N. R., Göttlicher S. G., Hal M., Wallin G., Richter A., Högberg P. (2007) No diurnal variation in rate or carbon isotope composition of soil respiration in a boreal forest. Tree Physiology, 27, 749–756.

Boone R. D., Nadelhoffer K. J., Canary J. D., Kaye J. P. (1998) Roots exert a strong influence on the temperature sensitivity of soil respiration. Nature, 396, 570–572.

Bowling D. R., Pataki D. E., Randerson J. T. (2008) Carbon isotopes in terrestrial ecosystem pools and CO_2 fluxes. New Phytologist, 178, 24–40.

Drewitt G., Wagner-Riddle C., Warland J. (2009) Isotopic CO_2 measurements of soil respiration over conventional and no-till plots in fall and spring. Agricultural and Forest Meteorology, 149, 614–622, doi: 10.1016/j.agrformet.2008.10.009.

Ehleringer J. R., Buchmann N., Flanagan L. B. (2000) Carbon isotope ratios in belowground carbon cycle processes. Ecological Applications, 10 (2), 412–422.

Ekblad A., Högberg P. (2001) Natural abundance of ^{13}C in CO_2 respired from forest soils reveals speed of link between tree photosynthesis and root respiration. Oecologia, 127, 305–308.

Fang C., Moncrieff J. B. (1996) An improved dynamic chamber technique for measuring CO_2 efflux from the surface of soil. Functional Ecology, 10 (2), 297–305.

Flanagan L. B., Kubien D. S., Ehleringer J. R. (1999) Spatial and temporal variation in the carbon and oxygen stable isotope ratio of respired CO_2 in a boreal forest ecosystem. Tellus, 51B, 367–384.

Griffis T. J., Baker J. M., Sargent S. D., Tanner B. D., Zhang J. (2004) Measuring field-scale isotopic CO_2 fluxes with tunable diode laser absorption spectroscopy and micrometeorological techniques. Agricultural and Forest Meteorology, 124, 15–29, doi:10.1016/j.agrformet.2004.01.009.

Hanson P. J., Wullschleger S. D., Bohlman S. A., Todd D. E. (1993) Seasonal and topographic patterns of forest floor CO_2 efflux from an upland oak forest. Tree Physiology, 13, 1–15.

Hanson P. J., Edwards N. T., Garten C. T., Andrews J. A. (2000) Separating root and soil microbial contributions to soil respiration: A review of methods and observations. Biogeochemistry, 48, 115–146.

Hesterberg R., Siegenthaler U. (1991) Production and stable isotopic composition of CO_2 in a soil near Bern, Switzerland. Tellus, 43B, 197–205.

Hutchinson G. L., Livingston G. P. (2002) Gas flux. In: Methods of Soil Analysis: Part 1, Physical Methods, edited by Dane J. H., Topp G. C., 3rd edition. Soil Science Society of America, Madison, 1159–1182.

Kayler Z. E., Sulzman E. W., Rugh W. D., Mix A. C., Bond B. J. (2010) Characterizing the impact of diffusive and advective soil gas transport on the measurement and interpretation of the isotopic signal of soil respiration. Soil Biology and Biochemistry, 42, 435–444.

Keeling C. D. (1958) The concentrations and isotopic abundances of atmospheric carbon dioxide in rural areas. Geochimica et Cosmochimica Acta, 13, 322–334.

Kutsch W. L., Staack A., Wötzel J., Middelhoff U., Kappen L. (2001) Field measurements of root respiration and total soil respiration in an alder forest. New Phytologist, 150, 157–168.

Kuzyakov Y. (2006) Sources of CO_2 efflux from soil and review of partitioning methods. Soil Biology and Biochemistry, 38, 425–448.

Lin G., Ehleringer J. R., Rygiewicz P. T., Johnson M. G., Tingey D. T. (1999) Elevated CO_2 and temperature impacts on different components of soil CO_2 efflux in Douglas-fir terracosms. Global Change Biology, 5, 157–168.

Marron N., Plain C., Longdoz B., Epron D. (2009) Seasonal and daily time course of the ^{13}C composition in soil CO_2 efflux recorded with a tunable diode laser spectrophotometer (TDLS). Plant Soil, 318, 137–151, doi:10.1007/s11104-008-9824-9.

Maseyk K., Wingate L., Seibt U., Ghashghaie J., Bathellier C., Almeida P., Lobo de Vale R., Pereira J.S.,Yakir D., Mencuccini M. (2009) Biotic and abiotic factors affecting the $\delta^{13}C$ of soil-respired CO_2 in a Mediterranean oak woodland. Isotopes in Environmental and Health Studies, 45 (4), 343–359.

Midwood A. J., Thornton B., Millard P. (2008) Measuring the ^{13}C content of soil-respired CO_2 using a novel open chamber system. Rapid Communication in Mass Spectrometry, 22, 2073–2081.

Miller J. B., Yakir D., White J. W. C., Tans P. P. (1999) Measurement of $^{18}O/^{16}O$ in the soil-atmosphere CO_2 flux. Global Biogeochemical Cycles, 13, 761–774.

Mortazavi B., Prater J. L., Chanton J. P. (2004) A field-based method for simultaneous measurements of the $\delta^{18}O$ and $\delta^{13}C$ of soil CO_2 efflux. Biogeosciences, 1, 1–9.

Ngao J., Epron D., Brechet C., Granier A. (2005) Estimating the contribution of leaf litter decomposition to soil CO_2 efflux in a beech forest using ^{13}C-depleted litter. Global Change Biology, 11, 1768–1776, doi: 10.1111/j.1365-2486.2004.01014.x.

Nickerson N., Risk D. (2009a) Keeling plots are non-linear in non-steady state diffusive environments. Geophysical Research Letters, 36, L08401, doi:10.1029/2008GL036945.

Nickerson N., Risk D. (2009b) A numerical evaluation of chamber methodologies used in measuring the $\delta^{13}C$ of soil respiration. Rapid Communication in Mass Spectrometry, 23, 2802–2810.

Nickerson N., Risk D. (2009c) Physical controls on the isotopic composition of soil-respired CO_2. Journal of Geophysical Research, 114, G01013, doi:10.1029/2008JG000766.

Ohlsson K. E. A. (2009) Reduction of bias in static closed chamber measurement of $\delta^{13}C$ in soil CO_2 efflux. Rapid Communication in Mass Spectrometry, 24, 180–184.

Ohlsson K. E. A., Bhupinderpal S., Holm S., Nordgren A., Lovdahl L., Högberg P. (2005) Uncertainties in static closed chamber measurements of the carbon isotopic ratio of soil-respired CO_2. Soil Biology and Biochemistry, 37, 2273–2276.

Plain C., Gerant D., Maillard P., Dannoura M., Dong Y., Zeller B., Priault P., Parent F., Epron D. (2009) Tracing of recently assimilated carbon in respiration at high temporal resolution in the field with a tuneable diode laser absorption spectrometer after in situ $^{13}CO_2$ pulse labelling of 20-year-old beech trees. Tree Physiology, 29, 1433–1445, doi:10.1093/treephys/tpp072.

Powers H. H., Hunt J. E., Hanson D. T., McDowell N. G. (2010) A dynamic soil chamber system coupled with a tunable diode laser for online measurements of $\delta^{13}C$, $\delta^{18}O$, and efflux rate of soil-respired CO_2. Rapid Communication in Mass Spectrometry, 24, 243–253.

Pumpanen J., Kolari P., Ilvesniemi H., Minkkinen K., Vesala T., Niinisto S., Lohila A., Larmola T., Morero M., Pihlatie M., Janssens I., Yuste J., Grunzweig J., Reth S., Subke J. A., Savage K., Kutsch W., Ostreng G., Ziegler W., Anthoni P., Lindroth A., Hari P. (2004) Comparison of different chamber techniques for measuring soil CO_2 efflux. Agricultural and Forest Meteorology, 123, 159–176, doi:10.1016/j.agrformet.2003.12.001.

Risk D., Kellman L. (2008) Isotopic fractionation in non-equilibrium diffusive environments. Geophysical Research Letters, 35, L02403, doi:10.1029/2007GL032374.

Takle E. S., Massman W. J., Brandle J. R., Schmidt R. A., Zhou X., Litvina I. V., Garcia R., Doyle G., Rice C.W. (2004) Influence of high-frequency ambient pressure pumping on carbon dioxide efflux from soil. Agricultural and Forest Meteorology, 124, 193–206, doi:10.1016/j.agrformet.2004.01.014.

Tans P. (1998) Oxygen isotopic equilibrium between carbon dioxide and water in soils. Tellus, 50B, 163–178.

Torn M. S., Davis S., Bird J.A., Shaw M. R., Conrad M. (2003) Automated analyses of $^{13}C/^{12}C$ ratios in CO_2 and dissolved inorganic carbon for ecological and environmental applications. Rapid Communication in Mass Spectrometry, 17, 2575–2582.

Tuzson B., Zeeman M. J., Zahniser M. S., Emmenegger L. (2008a) Quantum cascade laser based spectrometer for in situ stable carbon dioxide isotope measurements. Infrared Physics and Technology, 51, 198–206, doi:10.1016/j.infrared.2007.05.006.

Tuzson B., Mohn J., Zeeman M. J., Werner R. A., Eugster W., Zahniser M. S., Nelson D. D., McManus J. B., Emmenegger L., (2008) High precision and continuous field measurements of $\delta^{13}C$ and $\delta^{18}O$ in carbon dioxide with a cryogen-free QCLAS. Applied Physics B. Lasers – Optics 92, 451–458, doi:10.1007/s00340-008-3085-4.

Werle P., Mucke R., Slemr F. (1993) The limits of signal averaging in atmospheric trace-gas monitoring by tunable diode-laser absorption spectroscopy (TDLAS). Applied Physics B – Lasers O 57, 131–139.

Werner R. A., Brand W. A. (2001) Referencing strategies and techniques in stable isotope ratio analysis. Rapid Communication in Mass Spectrometry, 15, 501–519.

Wingate L., Ogée J., Burlett R., Bosc A., Devaux M., Grace J., Loustau D., Gessler A. (2010) Photosynthetic carbon isotope discrimination and its relationship to the carbon isotope signals of stem, soil and ecosystem respiration. New Phytologist, doi: 10.1111/j.1469-8137.2010.03384.x.

Zhang J., Quay P. D., Wilbur D. O. (1995) Carbon isotope fractionation during gas-water exchange and dissolution of CO_2. Geochimica et Cosmochimica Acta, 59, 107–114.

Zobitz J. M., Keener J. P., Schnyder H., Bowling D. R. (2006) Sensitivity analysis and quantification of uncertainty for isotopic mixing relationships in carbon cycle research. Agricultural and Forest Meteorology, 136, 56–75, doi:10.1016/j.agrformet.2006.01.00

"In the theory, the theory and the practice are the same, but in the practice…"

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Kammer A., Schmidt M. W. I., Hagedorn F. (2011) Decomposition pathways of ^{13}C-depleted leaf litter in forest soils of the Swiss Jura. Biogeochemistry, doi: 10.1007/s10533-011-9607-x.

Kammer A., Tuzson B., Emmenegger L., Knohl A., Mohn J., Hagedorn F. (2011) Application of a quantum cascade laser-based spectrometer in a closed chamber system for real-time $\delta^{13}C$ and $\delta^{18}O$ measurements of soil-respired CO_2. Agricultural and Forest Meteorology, 151, 39–48.

Walthert L., Graf U., **Kammer A.**, Luster J., Pezzotta D., Zimmermann S., Hagedorn F. (2010) Determination of organic and inorganic carbon, $\delta^{13}C$, and nitrogen in soils containing carbonates after acid fumigation with HCl. Journal of Plant Nutrition and Soil Science, 173, 207–216.

Kammer A., Hagedorn F., Shevchenko I, Leifeld J., Guggenberger G., Goryacheva T., Rigling A., Moiseev P. (2009) Treeline shifts in the Ural mountains affect soil organic matter dynamics. Global Change Biology, 15, 1570 –1583.

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